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MECHANISMS OF MICROPOLLUTANT ELIMINATION IN VERTICAL FLOW CONSTRUCTED WETLANDS

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Men love to wonder, and that is the seed of science.

Ralph Waldo Emerson

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Abstract

One of the biggest global challenges is the enormous growth of the population. With the growing population rises consequentially production and release of anthropogenic compounds, which then, due to insufficient wastewater treatment system, become pollutants, more precisely micropollutants (MPs). Advanced wastewater technologies presented in this dissertation are solutions applied for targeted elimination of MPs. Ozonation and adsorption on Activated Carbon or their combination belong to the most used advanced wastewater treatment technologies in Europe, however, they are suited for effluents of larger wastewater treatment plants. Therefore, an attempt has been made to test Constructed Wetlands (CWs) as an advanced wastewater treatment technology for small-to-medium sized WWTPs, which are typical for rural areas at the catchment of the river Sûre, the geographical border between Luxembourg and Germany. The efficiency of the CWs for the removal of 27 selected compounds has been tested at different scales (laboratory to pilot) in the Interreg Greater Region project EmiSûre 2017-2021 (Développement de stratégies visant à réduire l'introduction de micropolluants dans les cours d'eau de la zone transfrontalière germanoluxembourgeoise). The results of the project confirmed high ability of CWs to remove MPs from municipal effluents. The quantification of the main mechanisms contributing to the elimination of MPs within the CWs was thus established as the main target of the present PhD research, given the evidence of their high ability in the EmiSûre project. The main mechanisms have been identified as adsorption on the soil of the wetland, phytoremediation by the wetland macrophytes and bioremediation by the wetland microorganisms. The nature of the doctoral thesis is cumulative, the core of the thesis are the following four publications:

- Publication [I] describes the usage of CWs as a post-treatment step for municipal effluents.
- Publication [II] assesses the role of adsorption of the targeted MPs on the used substrates within the studied CWs and presents characterization of the wetland substrates.
- Publication [III] describes the role of the wetland macrophytes in the phytoremediation of the targeted MPs within the studied CWs. Furthermore, it reveals a comparison of the different macrophyte types in varying vegetation stadia.

- Publication [IV] outlines the role of the wetland microbes in the bioremediation of the targeted MPs within the studied CWs. Moreover, the wetland microbes known to be able to digest MPs or contribute to the elimination of MPs are identified and quantified.

Results suggest adsorption as leading removal mechanism (achieved average removal 18 out of 27 compounds >80%), followed by bioremediation (achieved average removal 18 out of 27 compounds >40%) and phytoremediation (achieved average removal 17 out of 27 compounds <20%).

The research described contributes to the extension of knowledge about CWs applied for the elimination of MPs from water. Some of the outcomes (deepened knowledge about soil influencing adsorption, recommendations for adjustment of operational parameters, etc.) could be used as a tool for enhancement of the wetland's treatment efficiency. The research is concluded by recommendations for further investigations of the individual mechanisms (e.g. application of artificial aeration or circulation of the reaction matrix could result in enhancement of bioremediation).

Kurzfassung

Eine der größten globalen Herausforderungen ist das enorme Wachstum der Bevölkerung. Mit der wachsenden Bevölkerung steigt die Produktion und Freisetzung von anthropogenen Substanzen, die dann aufgrund unzureichender Abwasserbehandlungssysteme als Schadstoffe, genauer gesagt als Mikroschadstoffe (MPs) wirken. Fortschrittliche Abwassertechnologien, die in dieser Dissertation vorgestellt werden, sind Lösungen zur gezielten Eliminierung von MPs. Ozonierung und Adsorption an Aktivkohle oder deren Kombination gehören zu den am häufigsten verwendeten fortschrittlichen Abwasserbehandlungstechnologien in Europa, eignen sich jedoch eher für Abwässer größerer Kläranlagen. Daher wurde versucht, Constructed Wetlands (CWs) als fortschrittliche Abwasserbehandlungstechnologie für kleine bis mittelgroße Kläranlagen zu testen, die typisch für ländliche Gebiete im Einzugsgebiet der Sauer, der geographischen Grenze zwischen Luxemburg und Deutschland, sind. Die Effizienz der CWs für die Elimination von 27 ausgewählten Mikroschadstoffen wurde im Interreg Greater Region Projekt EmiSûre 2017-2021 (Développement de stratégies visant à réduire l'introduction de micropolluants dans les cours d'eau de la zone transfrontalière germano-luxembourgeoise) auf verschiedenen Skalenniveaus (Labor- bis Pilotmaßstab) getestet. Die Ergebnisse des Projekts bestätigten die hohe Effizienz von CWs, Mikroschadstoffe aus kommunalen Abwässern zu entfernen. Die Quantifizierung der Hauptmechanismen, die zur Eliminierung dieser Mikroschadstoffe innerhalb der CWs beitragen, wurde somit als Hauptziel der gegenwärtigen Doktorarbeit festgelegt, da ihre hohe Leistungsfähigkeit bereits im EmiSûre-Projekt nachgewiesen wurde. Als Hauptmechanismen wurden Adsorption am Substrat des Wetlands, Phytoremediation durch die Feuchtgebietsmakrophyten sowie Bioremediation durch Mikroorganismen identifiziert. Die Ausgestaltung der Doktorarbeit ist kumulativ, der Kern der Arbeit sind die folgenden vier Publikationen:

- Die Publikation [I] beschreibt die Verwendung von CWs als Nachbehandlungsschritt für kommunale Abwässer.

- Die Publikation [II] bewertet die Rolle der Adsorption der Zielstoffe auf den verwendeten Substraten auf Basis der untersuchten CWs und stellt die Charakterisierung der Feuchtgebietssubstrate vor.
- Die Publikation [III] beschreibt die Rolle von Feuchtgebietsmakrophyten bei der Phytoremedation der ausgewiesenen Wirkstoffe innerhalb der untersuchten CWs. Darüber hinaus zeigt es einen Vergleich verschiedener Makrophytentypen in unterschiedlichen Vegetationsstadien.
- Die Publikation [IV] skizziert die Rolle der Feuchtgebietsmikroben innerhalb des Prozesses der Bioremediation zum Abbau der Zielsubstanzen in den untersuchten CWs. Darüber hinaus werden die Feuchtgebietsmikroben, von denen bekannt ist, dass sie in der Lage sind ausgewählte Zielsubstanzen aufzunehmen oder zur Eliminierung dieser beizutragen, identifiziert und quantifiziert.

Die Ergebnisse deuten darauf hin, dass die Adsorption der dominierende Entfernungsmechanismus ist (durchschnittliche Elimination von 18 der 27 Substanzen erreicht > 80%), gefolgt von der Bioremediation (durchschnittliche Entfernung von 18 von 27 Substanzen >40%) und Phytoremediation (durchschnittliche Entfernung von 17 von 27 Substanzen erreicht <20%).

Die beschriebene Forschung trägt zur Erweiterung des Wissens über CWs bei, die zur Eliminierung von Zielsubstanzen aus dem Wasser eingesetzt werden. Einige der Ergebnisse (vertieftes Wissen über die Adsorption des Bodens, Empfehlungen zur Anpassung der Betriebsparameter usw.) könnten als Instrument zur Verbesserung der Behandlungseffizienz von CWs verwendet werden. Die Forschung wird durch Empfehlungen für weitere Untersuchungen zu den einzelnen Mechanismen abgeschlossen (z.B. die Anwendung künstlicher Belüftung oder die Zirkulation der Reaktionsmatrix könnte zu einer Verbesserung der Bioremediation führen).

Contents

List of abbreviations

AC	activated carbon
ADS	adsorption
AGE	Administration de la Gestion de l’Eau
AMF	arbuscular mycorrhizal fungi
AMPA	aminomethylphosphonic acid
AOPs	advanced oxidation processes
AWWT	advanced wastewater treatment
BET	Brunauer, Emmett and Teller (specific surface area)
BIOCOS	biological combined system
BIOR	bioremediation
BOD	biochemical oxygen amount
BPB	bioremediation roots *Iris*
BPC	bioremediation roots *Phragmites*
BPNB	bioremediation new roots *Iris*
BPNC	bioremediation new roots *Phragmites*
BRA	bioremediation rhizosphere *Lythrum*
BRB	bioremediation rhizosphere *Iris*
BRC	bioremediation rhizosphere *Phragmites*
c_0	concentration of the dye in the initial solution
CAS	chemical abstract service
c_e	concentration of the dye at equilibrium
COD	chemical oxygen demand
CSO	combined sewer overflow
CWs	constructed wetlands
DO	dissolved oxygen
DOM	dissolved organic matter
E	oxidation-reduction potential

EBCT	empty bed contact time
EC	European Commission
EC	electrical conductivity (analytical methods)
EQSs	environmental quality standards
ESI	electrospray ionization
EU	European Union
FWS	free water surface
GAC	granulated activated carbon
HLB	hydrophilic lipophilic balanced
HLR	hydraulic loading rate
HRT	hydraulic retention time
HSSF	horizontal subsurface flow
HSV	hourly space velocity
IF	impact factor
K	adsorption coefficient
k_1	pseudo-first order rate constant
k_2	pseudo-second order rate constant
K_F	parameter corresponding to adsorption intensity in Freundlich equation
K_L	parameter corresponding to adsorption intensity in Langmuir equation
K_{LF}	parameter corresponding to adsorption intensity in Langmuir-Freundlich equation
KomS	Kompetenzzentrum Spurenstoffe
Kow	octanol/water partition coefficient
LC	liquid chromatography
LED	light-emitting diode
LCSB	Luxembourg Centre for Systems Biomedicine
LIST	Luxembourg Institute of Science and Technology
M	amount of the adsorbent used
MCPP	mecoprop
MPs	micropollutants
MRM	multiple reaction monitoring

MS	mass spectrometry
n	adsorption coefficient
NBS	nature-based solutions
NH_4-N	ammonium nitrogen
NO_3-N	nitrate nitrogen
OLR	organic loading rate
ORP	oxidation-reduction potential
PAC	powdered activated carbon
PE	population equivalents
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PHR	phytoremediation
pka	dissociation constant
PO4-T	total phosphate
q	amount of adsorbate per g of adsorbent
QB	flow rate coming into the bed
qe	adsorbate uptake at equilibrium
q_m	maximum adsorption capacity according to Langmuir and Langmuir-Freundlich models
qt	adsorbate uptake at time t
R_2	regression coefficient (square of the Pearson Product-Moment Correlation Coefficient)
R.r.	removal rate
RAC	regenerated activated carbon
RFID	radio-frequency identification
ROL	radial oxygen loss
RPM	revolutions per minute
rRNA	ribosomal ribonucleic acid
SPE	solid phase extraction
spp.	species pluralis (multiple species)
Std	standard deviation
SIDEN	Syndicat Intercommunal de Depollution des Eaux Residuaires du Nord

SJR	Scimago Journal & Country Rank
TCPP	tris(1-chloro-2-propyl)phosphate
TCIPP	tris(1-chloro-2-propyl)phosphate
TN	total nitrogen
TP	total phosphorus
TSS	total suspended solids
UN	United Nations
V	volume of the solution
V_B	volume of the bed
VF	vertical flow
VFCW(s)	vertical flow constructed wetland(s)
WFD	Water Frame Directive
WWTP(s)	wastewater treatment plant(s)

1 General introduction

The presence of micropollutants (MPs) (sometimes also called as microcontaminants or emerging contaminants) is creating serious challenges since these compounds are not being sufficiently removed by current conventional wastewater treatment plants (WWTPs) and therefore enter the water bodies through the municipal effluent [1,2]. This situation is getting more serious in the last decades since the MPs are of anthropogenic origin and so their amount is rising equally with the growth and aging of the global population.

As the term ‘micropollutants’ suggests, these compounds are occurring in water bodies in very low concentrations (ranges of ng/l - µg/l [1,3,4]). Many of the MPs are persistent (such as fluorosurfactants) and bioaccumulative (polychlorinated biphenyls, polycyclic aromatic hydrocarbons [5]). The application range of the compounds is very heterogeneous (pharmaceuticals, pesticides, personal care products, surfactants, etc.) and some of the compounds are, due to the mentioned wide applications, ubiquitous. There have been many studies confirming negative effects of MPs presence in water environment to aquatic fauna and flora (mortality of fish embryos, reduced ability of fish to swim, decreased heart rates of fish, feminization of male fish, decreased diversity of zooplankton, decrease of the richness of invertebrate families, etc.) [6–9]. Also, the presence of MPs in water may have negative impacts on human health (genotoxicity, tumors, aging, etc.) [10–12] and it contributes to the antibiotic resistance [13] or to inducement of mutagenicity [14]. Hence, European Commission (EC) took necessary steps regarding mandatory monitoring of these compounds (e.g. pharmaceuticals such as diclofenac or antibiotics clarithromycin, ciprofloxacin and erythromycin).

All European Union (EU) and United Nations (UN) member states declared to preserve the ecological and chemical status of the surface bodies by 2027 and agreed on achieving of Sustainable Development Goals of 2030 Agenda [15–19].

The Grand-Duchy of Luxembourg released the first regulation on implementation of the forth wastewater treatment step (the MPs cannot be/are not well removed in the conventional processes of primary, secondary and tertiary treatment) in July 2020, following the pattern of foreign countries (Germany (federal states North Rhine-Westphalia and Baden-Württemberg) or Switzerland). One of the main goals of the Luxembourg's national strategy is elimination of each of the selected substances (diclofenac, carbamazepine, clarithromycin and benzotriazole) by at least 80% (elimination measured from the influent and effluent of the additional step). Diclofenac and benzotriazole are compounds regulated in Germany and Switzerland as control compounds for the efficiency of the quaternary wastewater treatment [20]. Carbamazepine acts as a 'conservative tracer', as this compound is very recalcitrant and appears to be resistant to sorption and bioremediation [21], moreover, a quality standard for carbamazepine is prescribed in Luxembourg since 2016 in relation to the status of the surface water bodies. Antibiotic clarithromycin was selected, due to its aquatic ecotoxicity and other negative effects on the living organisms, to be a priority compound in the Water Frame Directive (WFD) [22] and is also being analyzed in Luxembourg rivers Alzette and Sûre. These four compounds belong to the targeted compound composition of the key PhD project, all the compounds will be introduced later in the work. On the Fig. 1.1 are shown WWTPs of Luxembourg, which are considered for update by the fourth wastewater treatment step.

In this dissertation are briefly outlined implementations of the fourth wastewater treatment step to WWTPs Reisdorf and Echternach.

The technological target of the general MP strategy is then clear – development and successful application of technologies, which are able to remove these substances before entering the water bodies. The challenge of this target lies in a fact that besides the mentioned matters of concern, such as low concentration, persistency, bioaccumulation and heterogeneity of the MPs, the technologies of forth treatment step applied until now often have drawbacks, which could be limiting their usage. The mostly applied technologies are adsorption on activated carbon (powdered or granulated – PAC or GAC) and ozone. All the mentioned technologies are effective in removal of MPs, however, as these technologies require high cost and energy investments in long-term perspective, an attempt for finding a solution for regional rural areas in Luxembourg for small-to-medium WWTPS (<20 000 population equivalents (PE)) has been made. For this, constructed wetlands (CWs) are considered as a solution for the quaternary treatment of wastewater in Luxembourg with the aim to decrease emissions of MPs in the common rural areas [23]. An overview of the most common advanced wastewater treatment (AWWT) technologies with the aim to remove micropollutants is drawn in chapter 2.1.1. In the sub-chapter 2.1.2. are drawn some of the deficiencies and in the following chapter, 2.1.3. are introduced CWs as an AWWT technology, which is representing many solutions for the discussed drawbacks, and which is also main target of this research. The objective of the PhD research is to examine and to evaluate application of CWs as a solution for removal of MPs from wastewater effluents in small rural areas of Luxembourg. Moreover, a substantial focus is given on mechanisms which contribute to removal of MPs within the CWs and determination of the most dominant one.

2 State of the art

2.1.1 Presentation of the processes in quaternary wastewater treatment

In this chapter will be introduced the commonly used technologies and processes applied in the fourth wastewater treatment step aiming to eliminate MPs in the municipal effluents. An example of classification of these processes is shown in the Tab. 2.1.

Tab. 2.1. Examples of division of the processes applied for elimination of MPs.

Oxidation processes		**Adsorption processes**		**Physical separations**		**Others**
Ozonation	Advanced Oxidation Processes (AOP)	Powdered Activated Carbon (PAC)	Granular Activated Carbon (GAC)	Nanofiltration	Reverse osmosis	Photodegradation processes

2.1.1.1 Ozonation

Ozonation is an AWWT technology, which is widely used for elimination of MPs from municipal effluents. It has been introduced to water treatment more than 120 years ago [24], starting with applications in drinking water treatment and later also in wastewater treatment. The principle of ozonation consists in two reactions; the reaction of ozone with compounds containing electron-rich groups, where OH radicals are formed (HO•) and indirect reaction of the HO• with the targeted compound. The source for HO• is the effluent organic matter, which is than one of the most important parameters of ozonation. Ozonation is influenced by external parameters, such as pH, temperature and composition of the matrix or turbidity. Ozonation can be in some cases less effective than another commonly used AWWT technology - adsorption on activated carbon (AC), depending on the reactivity of ozone towards the targeted compound. For example, compounds such as primary amines, amides or nitro-derivates show low or no reactivity with ozone [25,26]. Also, ozone has low solubility in water and a short lifetime [27]. However, there are some cases, where ozonation is more effective than AC, e.g. in removing of estrogenicity from wastewater (ozonation 91.7%, AC 75%) [28].

2.1.1.2 Advanced oxidation processes

Besides ozonation, the processes of advanced oxidation (AOPs) are used for large scale as well, however, their usage is not that expanded. Similarly to ozonation, the AOPs were firstly used for treatment of drinking water around 1980 [29]. The principle of AOPs is oxidation process related to generation of hydroxyl radicals, which are than attacking the targeted compounds [30]. Due to their short lifetime, the hydroxyl radicals can be generated by different procedures. Combination of ozone with hydrogen peroxide was introduced as a first one (so-called peroxone-process) and is applied most thanks to its simple application in the industrial scale [31]. Other procedures include application of ultrasound, ultraviolet radiation (UV), or different catalysts, where the titanium dioxide belongs to the most frequent ones [32,33]. An example of the AOPs is the Fenton process, where the hydroxyl radicals are generated in presence of the Fenton's reagent (hydrogen peroxide with ferrous iron). The Fenton process underwent some modifications due to restricted operational conditions (pH ~ 3) and other processes have been introduced (electro-Fenton, photo-Fenton and heterogenous Fenton [34]). In electro-Fenton, the Fenton's reagent is produced by electrochemical procedures, in photo-Fenton, the system is exposed to the UV light aiming to enhance the photo-reduction of the ferrous and ferric ions, and in heterogeneous Fenton are used diverse catalysts in order to create the Fenton's reagent (clay materials, zeolites, metal-organic compounds) [35]. However, these AOPs are not used widely due to reasons further introduced in the following chapter (i.e. high energy demands, formation of harmful by-products, etc.)

2.1.1.3 Activated carbon

Adsorption on AC is a technology, which is representing broadly the AWWT processes and is applied in many full-scale applications. The principle of the process is adsorption of the substances on the surface of the AC [36]. Thanks to its properties such as: an extensive surface area, broad ability to create various surface interactions, high porosity and in general excellent adsorption capacity [37] is AC widely used for removal of MPs [38,39]. The efficiency of the AC to adsorb MPs depends on many factors; of course previously mentioned characteristics of the medium, but also nature of the adsorbates (hydrophobicity, charge, chemical structure, complexity, etc.). Besides these "internal" factors, the strength of adsorption is influenced by "external" factors as well (pH, composition and temperature of the wastewater) [1]. The AC is commonly applied in two forms – either powdered activated carbon (PAC) or granular activated carbon (GAC). PAC is often applied as a stand-alone contact reactor with the fluidized bed, followed by clarifier and employed directly after biological treatment as a post-treatment [40]. GAC is usually used as a monomedia adsorbent filling columns, whereas AC usage is in general higher in PAC applications for removal of MPs [41]. AC is one of the most used AWWT processes for removal of MPs in Europe [41] for bigger WWTPs (> 20 000 PE) [42,43]). When the saturation of the active surface of the AC is reached (depending e.g. on the character of the treated matrix and of the carbon material), the material needs to be replaced by a new one, or eventually by regenerated one (achieved by bioregeneration, electrochemical regeneration, microwaves, etc. [44–46]). The regeneration results than in decrease of the material's removal potential, but it has better carbon footprint and lower investments cots (about 30%). As mentioned previously, AC is often coupled together with ozonation, allowing than lower dosage of ozone resulting in much lower energy consumption [47]. Moreover, it has been demonstrated that AC can increase the transformation of ozone into the hydroxyl radicals [31]. Additionally, this combination can result in higher decrease of the MPs emissions by more than 80 %, as the application of ozone could overcome limitations caused of the AC adsorption capacity [25]. Also, the AC can remove hazardous degradation products of ozonation, which makes this coupled technology more sustainable [48].

2.1.1.3.1 Application of ozonation and activated carbon in Europe

A very recent study [49] evaluated toxicity of in total 1337 compounds representing the estimation of MPs in European scale and assessed an equivalent of the current toxicity to non-treated wastewater discharge of 160 million people (about 30% of the wastewater produced within the EU). This study stated that with introduction of the AWWT processes at big WWTPs (>100 000 PE) this number could be reduced to 95 million. These data describe well the current situation in Europe and stress the need of launch of the AWWT processes aiming to reduce the MPs emissions in the water bodies. Becoming a front runner within the European countries, Switzerland implemented upgrades for MPs removal to 123 out of 750 WWTPs [28]. Besides Switzerland, AC and ozonation are technologies mainly used in other European states, such as Germany, Sweden, the Netherlands, Denmark and Belgium [50,51]. In the Fig. 2.1. is shown map of Switzerland with illustrated upgraded WWTPs by AC, ozonation and combined processes.

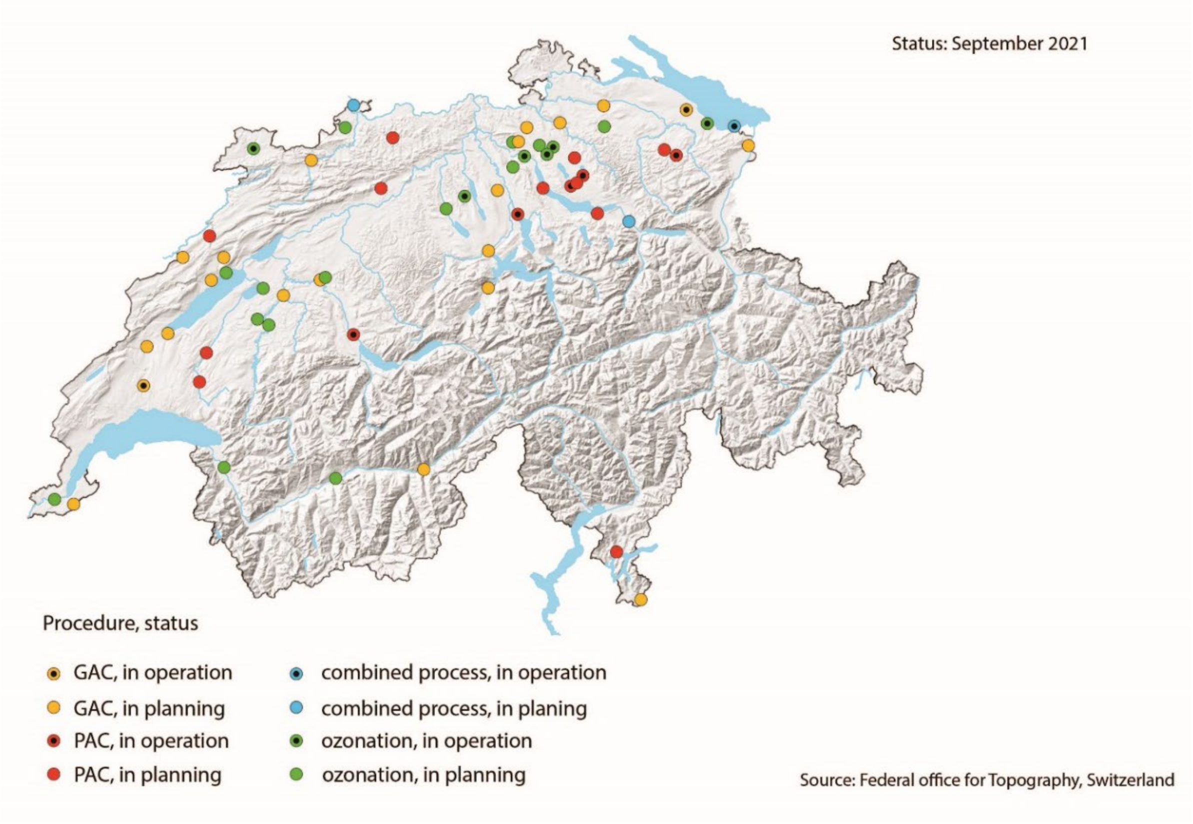


Fig. 2.1. Map of Switzerland with illustrated upgraded WWTPs by AWWT processes (AC, ozonation and combined processes of ozonation + GAC, ozonation + PAC or PAC + GAC [52]).

Similarly, the German federal state Baden-Württemberg already upgraded some of its WWTPs with integrated AC processes for removal of MPs, as appeared in Fig. 2.2.

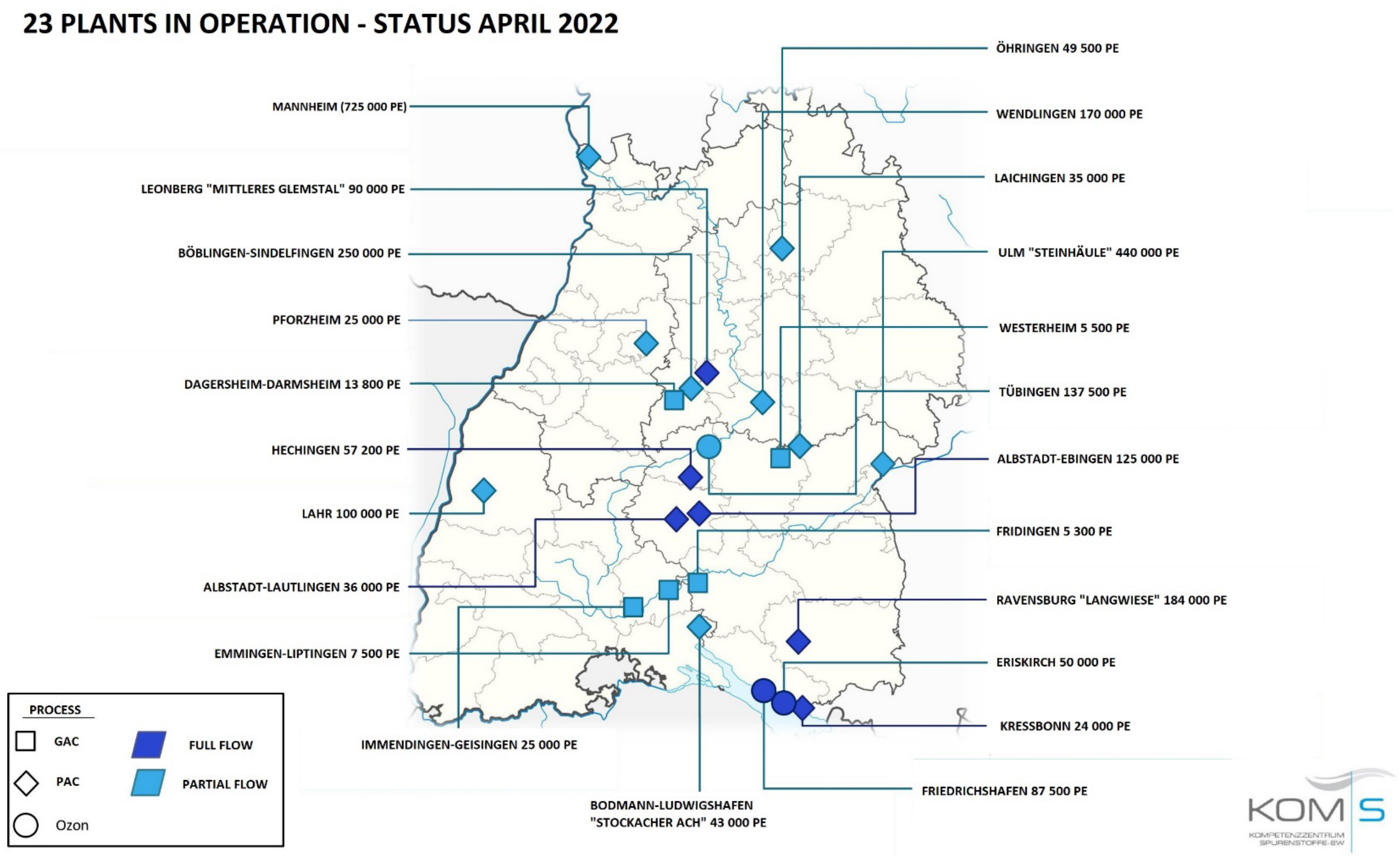


Fig. 2.2. Map of Baden-Württemberg with illustrated upgraded WWTPs with the advanced processes [53].

2.1.1.4 *Physical separations*

Mostly used processes in the membrane separations are nanofiltration and reverse osmosis [54,55]. The basic principle of these processes lies in the ability of the membranes to reject the MPs. The materials of the membranes are varying, most commonly used materials are different types of polymeric films (e.g.polyamide). The key parameters influencing the rejection's strength are the size of the effective membrane area, the selectivity of the membrane (often determined by pore distribution), the charge of the surface, the volumetric flow and of course, as mentioned previously, the nature of the treated matrix [56–58]. Additionally, the physical separation processes are able to remove antibiotic resistant genes and bacteria and thus to contribute to decrease of the general antibiotic resistance, which is a secondary advantage of the antibiotic removal [59]. However, membranes are not widely used in big scale applications due to frequently occurring fouling, thus usage of membranes in big scale would require much bigger membrane surface area and increased implementation and operation costs [60,61].

2.1.1.5 Photodegradation processes

Photodegradation by UV is a method commonly used for elimination of contaminants present in water, in general there are two types of this process. The first type is direct photodegradation – the degradation occurs due to adsorption of photons by the targeted compound [62]. The second type is indirect photodegradation – the degradation occurs due to reaction of the targeted compound with a reactive agents created by photosensitizers (dissolved organic matter (DOM) or nitrate from wastewater), which can absorb radiation in order to reach the excited state [63]. As the degradation of compounds by direct photodegradation is often limited from various reasons (e.g. adsorption spectrum of the targeted compound, the quantum yield of the photochemical process needs to be reasonably large, disturbance of the matrix background), the modification by e.g. pH can result in faster photodegradation of the targeted compounds (however, depending on the chemical structure of the compounds, their pKa, etc.) [64].

The effectivity of the mentioned technologies is demonstrated on example of elimination/rejection of diclofenac (Tab 2.2.)

Tab 2.2. Demonstration of removal abilities of various AWWT technologies.

Technology	Removal/rejection of diclofenac achieved (%)	Reference
Ozonation	73	[65]
Ozone coupled with H_2O_2	89	[66]
Fenton process	98	[67]
Electro-Fenton process	97.8	[68]
Photo-Fenton process	97	[69]
Heterogeneous Fenton process	97	[70]
PAC	90	[71]
GAC	99.7	[72]
Nanofiltration	99	[56]
Photodegradation	95.8	[73]

2.1.2 Limitations of the processes in quaternary wastewater treatment

Previously mentioned technologies, even though some broadly used, have numerous disadvantages. These flaws will be introduced by bullet points in this chapter.

2.1.2.1 Ozonation and AOPs

- Implementation of ozonation units in the largest scales is not realistic because of its very high application costs (piping – capital costs of generators, ozone generation systems and contractors, in-plant pumping, construction costs, installation, and others [74]).
- The oxidants for AOPs require high cost requirements and the chemical activators (transition metal catalysts) for oxidations can pose a risk of secondary contamination [75].
- For UV combined applications speak in their disadvantage very high operation costs (75.38 $/m^3) [76,77] and increased energy consumption by the artificial lamp.

- When the targeted compounds are found, their complete degradation by AOPs is often not possible [50].
- Catalyzed oxidations are limited by the active surface of the catalyst [78].
- Recovery and recycling of titanium dioxide as oxidant is very expensive [79].
- Use of persulfates as oxidants is resulting in increasing intrinsic toxicity [80].
- Fenton process has limitations for its usage under optimum conditions to pH ~ 3, neutralization requiring additional depuration and expensive treatment of Fe(III) hydroxide.
- Formation of potentially carcinogenic bromate as a disinfection by-product during ozonation of bromide-containing waters [81].
- As results of usage of AOPs can be produced various metabolites and by-products with toxicological impacts and low biodegradability [82–85], (as an example degradation of various pharmaceuticals by electro-, photo- and heterogeneous Fenton resulting in creation of by-products causing chronic toxicity during the development of spore-born gametophytes or degradation of sulfamethoxazole by photo-Fenton resulting in formation of by-products causing inhibition of activated sludge [86–88]).

2.1.2.2 *Activated carbon*

- The carbon materials are often produced in Asia and are then consequently transported over long distances to Europe [41].
- In European WWTPs is mostly used bituminous coal, which is related, due to its extraction, to environmental and social negative impacts. This type of coal is often produced from coconut shells, frequently coming from monocultures, where extensive use of chemical fertilizers and pesticides is needed [89].
- When using PAC, an additional step for separation of the PAC from wastewater is needed (mostly sand filtration or an ultrafiltration unit [90,91]). Implementation of these steps increases the cost requirements of the technology.

- GAC needs to be replaced regularly.
- Regeneration of both types of the AC, PAC and GAC, is very costly and has high energetical demands.

2.1.2.3 Physical separations

- One of the disadvantages of this technology lies in fact that the membrane needs to be cleaned repetitively and it often requires combination of other treatment processes (AOPs, bacterial reactors) to achieve the removal of the MPs.
- Frequent fouling of the membrane by the concentrate, resulting in shortening the membrane's stability and lifetime hinders application of nanofiltration in wastewater treatment for removal of MPs [92].
- Accumulation of salts, low water flux and accumulation of concentrated waste streams (called retentate) containing high levels of contaminants are the major disadvantages of reverse osmosis membrane reactors [93,94].
- In case of nanomembranes is the necessity to dispose retentates from this process for reclamation of secondary effluents of WWTPs [95].
- The choice of the nanofiber, e.g. polystyrene belongs to one of most widely used polymers for nanofiber production, however, its hydrophobicity limits its full use in advanced wastewater treatment processes.
- It is possible to enhance the removal efficiency of nanofibers by different types of e.g. photocatalysts, however, they can have drawbacks such as secondary contamination due to leaching or poor recovery [96].

2.1.2.4 Photodegradation processes

- MPs can be eliminated also by photodegradation, however, the compounds, which can be treated by this method, need to be photodegradable (e.g. photodegradable dyes).

- For the technology itself, it is not possible to use solar light as a source of the UV with the ability to lyse some compounds, therefore a need of a purchase of the UV source is required, which is often costly.
- Often must be induced proper wavelength of the UV source, which is increasing the financial requirements [97].
- The photodegradation is often not efficient as a process itself, therefore a need of additional catalyst (metal complexes, etc.) is required [98].
- Usage of photodegradation results in production of harmful by-products [88].

The described technologies can be used as quaternary step in wastewater treatment, mainly in context of bigger WWTPs (e.g. > 20 000 PE). As upgrade of the WWTPs for MPs elimination requires high long-term investments, nature-based solutions (NBS) – namely constructed wetlands in this case - can be introduced as a promising solution for small to medium sized WWTPs, as they require much lower cost investments.

2.1.3 Constructed wetlands

As the previous technologies are considered as intensive (due to the high costs and intensive operational energy demands), CWs are often labeled as extensive (low maintenance costs and better environmental, economic and social sustainability, however requiring higher area demands [99]). This technology, when used for quaternary treatment, is normally designed for effluents of small municipalities [23,99]. When looking at the disadvantages and lacks of the previously mentioned technologies, it is possible to consider CWs as a technology offering, if not solutions, at least minimization of some of the mentioned problems.

The development of general usage of CWs started in the 1950s' and since then it is rising rapidly [100]. CWs are engineered systems of artificial origin, however, they are mostly composed from natural components, such as: soil, plants, gravel and microorganisms naturally present in the listed elements.

Because of their natural composition, they are helping to conserve nature areas and they actively contribute to enhancement of biodiversity by e.g. application of broad variety of wetland plants. [101]. However, it is necessary to mention that the selected wetland plants need to fit well the application (as described later in chapter 2.1.3.1.1). The implementation costs are considerably reduced, than the costs of previously mentioned advanced wastewater treatment technologies [102]. Plus, once the wetland is established, the operational costs are very low, too [23]. Due to rapidly increasing urbanization is the need for nature restoration in cities becoming an urgent demand. CWs are providing solution also to this global problem, as they are often used as NBS for water treatment in urban areas (e.g. as added element in urban parks for water storage, stormwater management or ecosystem services provision).

From the above mentioned reasons were CWs proposed as a solution for reduction of the MPs in the rural areas of Luxembourg where small-to-medium WWTPs are mostly present. In this work, we focus on application of CWs for removal of MPs from municipal effluents. This topic has been targeted already in some previous studies [99,103–105], however, it still requires further investigations. There are several other domains, where can be CWs used (e.g. treatment of urban runoffs, contaminated groundwater, leachate, mine drainage, industrial effluents, greywater, rainwater, domestic and agricultural effluents) [102,106–110].

There are different types of CWs, depending on the aim of the CWs' usage, configuration and consequently on the adapted design. Common configuration types of CWs are shown in Fig. 2.3.

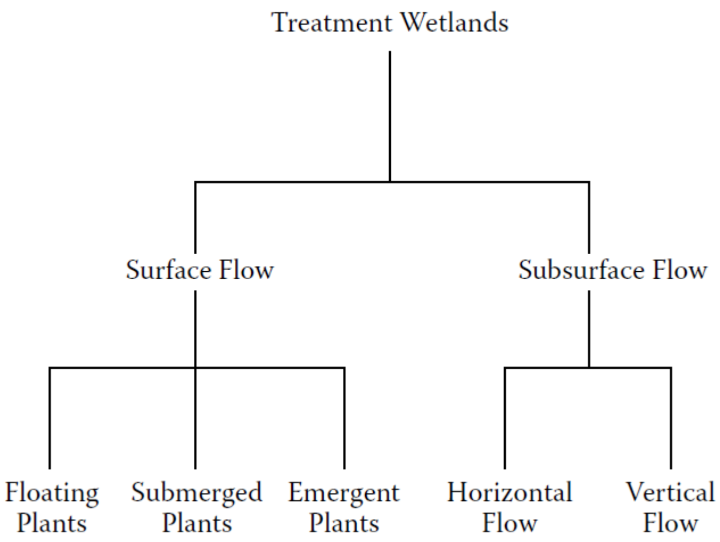


Fig. 2.3. Basic types of CWs [111].

Three types of wetlands, which are recently in a broad use, are:

- surface flow – sometimes called as free water surface (FWS) wetlands, which are similar in character to natural swamps (used mostly for water purification as an advanced treatment of effluent from secondary and tertiary wastewater treatment processes) [111];
- horizontal subsurface flow (HSSF) wetlands, where typically gravel is used for a bed medium, which is then planted. Under the surface of the bed is retained water, which flows horizontally from the inlet to the outlet The HSSF are typically used for secondary treatment of small systems or single-family houses, or for treatment of industry wastewaters [111]. The established anaerobic conditions enable removal of BOD (biochemical oxygen demand) and enhanced denitrification in HSSF wetlands, which does not take place under aerobic conditions present in vertical flow wetlands.
- vertical flow (VF) wetlands, which can employ a broad scale of the soil media acting as a filter. The surface of the VF wetland can be planted with various plants as well. As the name suggests, the water flows than vertically from the top of the wetland to the bottom and is delivered in

intermittent pulses, which ensures access of oxygen in the system. Therefore, VF wetlands have ability to oxidize ammonia in contrary to HSSF, and they have been widely applied in applications with higher ammonia levels, such as food processing of landfill leachates [111].

In this work are used vertical flow wetlands in subsurface configuration. Usage of vertical set-up for advanced wastewater treatment has numerous advantages compared to the other types of wetlands, such as:

1. lower tendency to clogging and overloading thanks to the vertical flow (if treating effluents of small WWTPS with COD (chemical oxygen demand) <50mg/l);
2. better oxygenation in unsaturated flow (aerobic conditions – crucial for removal of persistent MPs);
3. smaller area requirements in contrary to horizontal CWs.

These factors were considered when making the choice of the studied wetland's configuration, especially point 2. showed to be very important for removal of recalcitrant compounds which are removed mostly during aerobic conditions (e.g. carbamazepine and diclofenac).

In Fig. 2.4. is shown illustration of horizontal and vertical subsurface flow CWs.

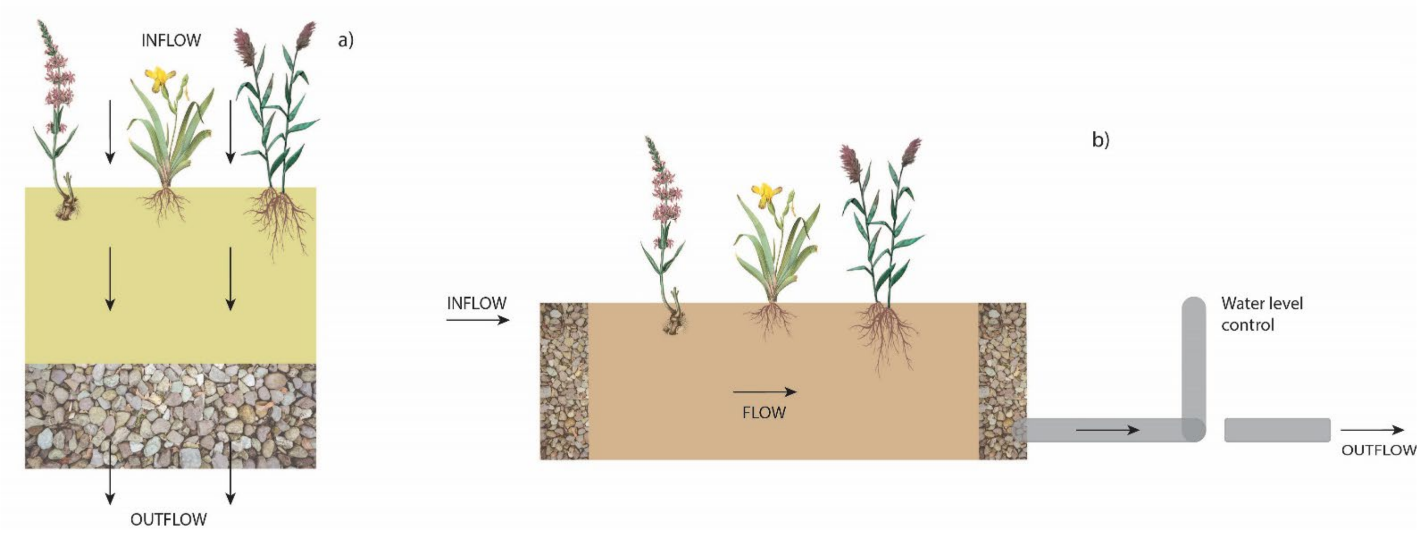


Fig. 2.4. Illustration of vertical (a) and horizontal (b) subsurface flow CWs.

2.1.3.1 Design and operational parameters of CWs in vertical flow configuration

VFCWs belong to the type of wetlands whose development started in the last decades (around 1990). At the beginning of the VFCWs design development it was supposed that plants are the main actors in the removal processes of the contaminants, however, this fact was later refuted [112] and it was found out that processes within all wetland media contribute to the removals.

The treatment efficiency of VFCW depends, as Fig. 2.4. suggests, on the type of used substrate and plants, on the plant stem density (higher planted wetlands result in higher removals of pollutants [113]), further than on operational conditions, such as: area of the wetland, water quality or hydraulic conditions (hydraulic loading rate (HLR) or hydraulic retention time (HRT)), resting time, mode of operation if alternating or parallel) and physicochemical parameters (temperature, pH and dissolved oxygen (DO)). These parameters need to be specifically chosen and designed based on the type of wastewater and on the desired type of treatment.

The factors influencing the effectivity of the treatment wetlands are described as follows:

2.1.3.1.1 Choice of the wetland media

Substrate

Substrate is a very important component in CWs and its role is even more relevant in subsurface configurations. Its relevance is due to multiple reasons: substrate provides basis for the plants, microorganisms and fungi growth and it acts as an adsorbent for the pollutants, which are aimed to be removed from water. When choosing a substrate for a CW, it is important to focus on some particular properties, which can contribute to the enhanced removal of MPs – these properties are mentioned further. By the choice of the substrate it is than possible to influence the pollutant removal process.

The treatment efficiency of the applied soil is influenced by numerous factors, such as active surface, surface charge (improved interaction with charged compounds), porosity, particle size, texture (influence of the HRT and therefore also removal of MPs), conductivity, or oxygen supply [114]. Oxygen can be provided by artificial aeration, eventually can have natural access to the wetland thanks to intermittent flow dosing (in case of VFCWs), or is provided by macrophytes via radial oxygen loss (ROL) – the plants can transport oxygen from photosynthesis to the roots and then release part of the oxygen to the rhizosphere [115]. ROL than creates aerobic conditions for the microbes which contribute to the removal of MPs. Furthermore, several studies showed importance of pH - improved adsorption capacity of the soil towards contaminants could be achieved when pH was lower than 7.4 [116].

One of the most frequently used substrates in CWs is gravel [102]. It proved to be successful when applied in wetlands used for the treatment of domestic wastewater [117] or for the removal of MPs [118–120]. Due to its bigger particle size, it is often used at the bottom of the subsurface installations to prevent clogging and avoid loss of the active substrate material, as it is applied in our subsurface wetland configuration. Sand shows good efficiency for removal of MPs, probably because of its higher relative hydraulic conductivity resulting in enhanced interactions between soil and the contaminants. Additionally it is also important to take into account, if possible, all properties and factors influencing the ability of the substrate and plants to withstand the conditions created by the wastewater effluent (concentrations of nutrients and pollutants, change of temperatures) and to eliminate the contaminants. Other used substrates are for example furnace slag, peat, clay and its aggregates, muck or different types of zeolite or activated carbons, eventually also different mixtures of conventional and unconventional substrates (e.g. sand with plant-based biochars). It is also possible to use combinations of different substrates – either in layers (each substrate is in individual layer) or in mixtures. Some of the studied wetland substrates in this work were used in mixtures, the reason for that was to evaluate the adsorption efficiency of the different homogeneous mixtures with changing ratios of the base substrate – sand and the admixtures (biochar and zeolite). Usage of the substrates depends mostly on their ability to adsorb pollutants, such as phosphorus, nitrogen, or MPs [121–123].

Plants

The most important parameter for the choice of a wetland plant is that the plant needs to be able to grow in aquatic environment. That is why most of the plants used in CWs are so-called macrophytes – aquatic plants. Another important parameters for the choice of wetland plants are: resistance of the plants against toxicological effects of the MPs, resistance to seasonal changes and adaptability to the changing character of the matrix (differing hydraulic conditions, fluctuating amount of nutrients and contaminants, etc.). Another very important parameter is the ability of the plants to up take the contaminants (metals, MPs, cyanotoxins) from the wastewater. When the pollutants are taken up by the plant, they are degraded by internal plant mechanisms in summary called as phytoremediation. The ability of the plants to up take the pollutants depends on multiple factors, the most important one is the hydrophobicity of the compounds. Compounds, which are lipophilic enough (0.5 – 3.5 log Kow) to be transferred through the membranes of the plant cells, are likely to be up taken by the plants [102]. Thanks to the roots growth through the profile of the wetland, the plants are naturally preventing the substrate media from clogging and additionally create routes that favor oxygenation. Another important role of the plants is to create advantageous surroundings for symbiotic relations with the wetland microorganisms – bacteria and fungi. Summary of the role of plants in CWs is illustrated in Fig. 2.5.

There are several plant species used widely in wetland applications, mostly common reeds, which are able to withstand changing conditions at outdoor wetlands. In Europe typical macrophytes are *Phragmites australis, Typha* and *Scirpus* spp., because of their many times proven ability to up take pollutants from water [124]. Macrophytes are contributing to the removal of pollutants from water by the previously mentioned ROL and phytoremediation. In the research discussed in this dissertation following macrophytes were used: (1) *Phragmites australis*, a plant which belongs to the most common ones in CW applications, because of its ability to create very long and quite strong roots, which are able to grow through the profile of the substrate. The shorter, but very dense and thin roots of (2) *Iris pseudacorus* complemented this property. Last plant, which was used in the studied wetlands was (3) *Lythrum salicaria*, a plant which is able to create strong symbiotic relationships with the soil microorganisms and therefore to contribute to enhanced removal of the MPs. Moreover, the used macrophytes are flowering in the summer season and therefore contribute to the enrichment of biodiversity.

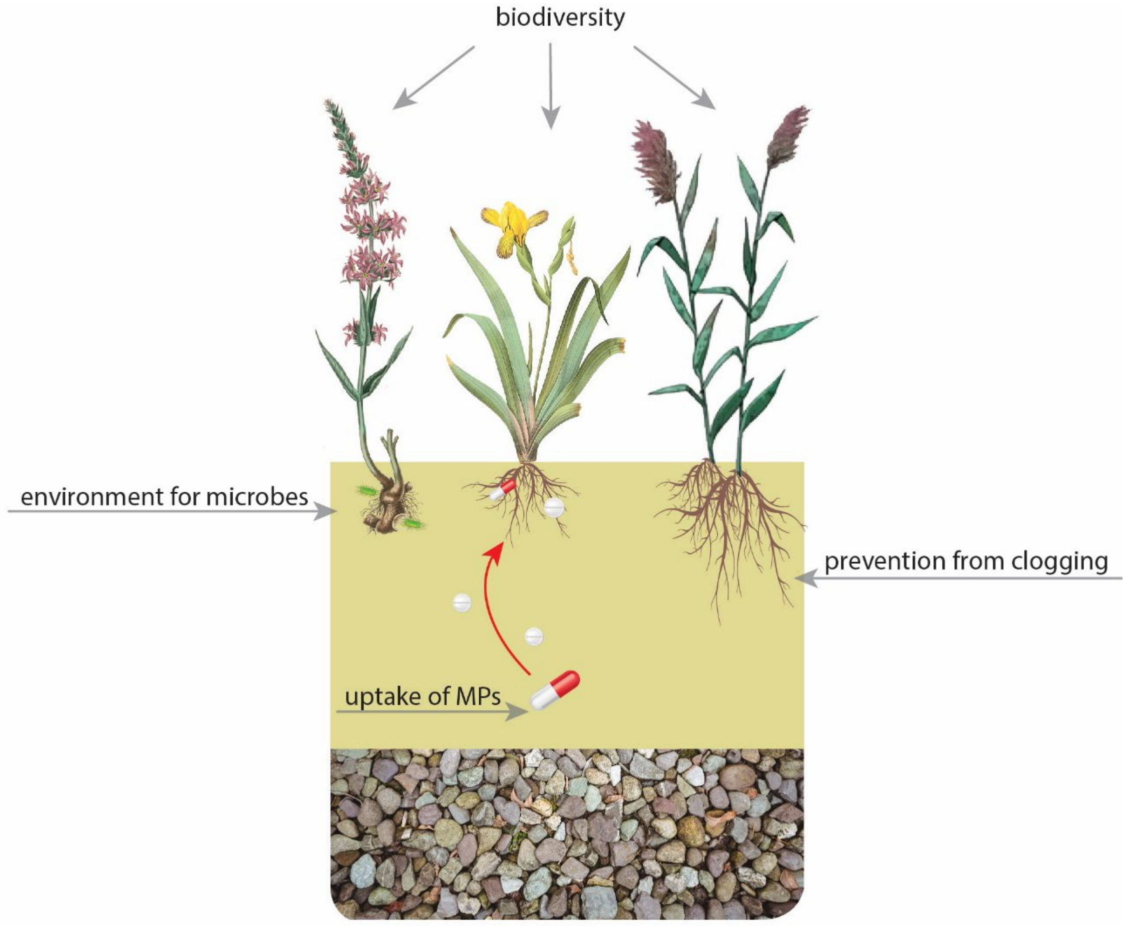


Fig. 2.5. Essential roles of plants in CWs.

Microorganisms

There is a broad variety of microorganisms present in the wetlands and they are contributing significantly to the removal of different types of pollutants (including MPs) from wastewater as well [125]. Microbes have typically first access to compounds, which are being dissolved in water and either can eliminate them directly or live in symbiosis with the plants and contribute than to phytoremediation [111]. The diversity and richness of the microorganisms is varying depending e.g. on the type of the treated wastewater, on the configuration and design of the wetland, on the nature of the substrate, etc. [125–127]. The removal ability of the plants and microorganisms is varying depending on the season – removal in warmer season, (in middle Europe from spring to autumn), is generally higher, than the removal during cold season.

Arbuscular mycorrhizal fungi (AMF) are soil-born microbes which are commonly present in CWs and contribute to the removal of nutrients and contaminants in an indirect way by their symbiotic relationship with the wetland macrophytes. This symbiotic relation is than resulting in enhanced phytoremediation. AMF are briefly studied also in this PhD research. Other studies proved AMF's inevitable contribution to the removal of MPs, such as diclofenac or ibuprofen [128]. The abundance of AMF depends on factors such as: flooding conditions, temperature or pH [129].

Last organisms, which might be important part of a wetland in terms of pollutants, are algae. Algae are important indicators of water purity, their presence is strongly dependent on light access, they can increase concentrations of DO via photosynthesis, and therefore to contribute to aerobic conditions preferable for removal of MPs [130]. There are evidences that algae have an impact on removal of nutrients (mostly ammonia and phosphate) and contaminants from wetlands and some recent studies are even discussing using algae and microalgae as independent advanced wastewater treatment technologies (in separate installations – algal ponds) [131–133]. In this work is role of algae in CWs not further discussed, because their existence is, as mentioned, dependent on light and therefore their occurrence in VF configurations is not abundant [111].

It is important to plan the choice of the substrate and plants for the whole wetland unit, because by the choice it is possible to influence the processes in the wetlands. However, the processes are occurring simultaneously, therefore it is difficult control each of them. From mentioned above it is clear, the soil, the plants and the microbes are the main actors contributing to removal of pollutants (in this case mostly focused on MPs) within the wetlands. Therefore, the main removal mechanisms can be summed-up as:

adsorption on the soil, phytoremediation by the plants and bioremediation by the microbes. The scheme of removal mechanisms within VFCW is shown in Fig. 2.6.

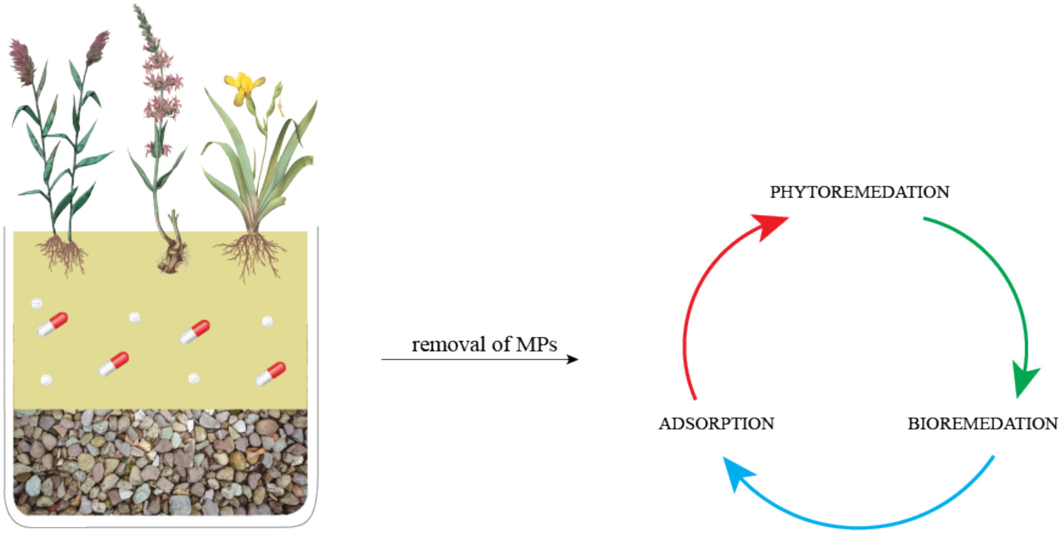


Fig. 2.6. Scheme of removal mechanisms within the VFCW.

2.1.3.1.2 Main design parameters of the wetland

Area of the wetland

There are couple of equations available for the calculation of the area of a VFCW [134], equation 2.1 is chosen as it is suitable for general calculation of the VFCW's area with help of so-called scaling factors [111]:

$$A = mPE^{b} \qquad \text{Eq. 2.1}$$

where

A = area of the required bed (m^2)

b = exponent

PE = population equivalent

m = scaling factor (HLR, HRT, etc.)

The choice of the parameters *m* and *b* depends on the wetland's parameters and requirements on its efficiency, e.g. number of the treated PE, type of pollutants, number of stages, hydraulic conditions etc. Another factor, which is crucial for sizing of the VFCW is secured oxygen availability and transfer, creating aerobic conditions in the wetland, which are important for removal of constituents such as BOD, COD and ammonium-nitrogen [135]. When sizing a VFCW, it is important to fulfill two criteria:

1. The wetland bed needs to enable the transfer of the wastewater through the bed before the next dose arrives, and in the same time it needs to provide enough contact time for the present dose to interact with the soil, the microbes and the plants to achieve required remediation.
2. The surface area needs to allow sufficient oxygen transfer to take place. [134]

From the reasons mentioned above, the VF systems are mostly dosed intermittently giving enough rest time between a cycle and the other and using percolation by gravity to increase the contact time, as it was in the case of the installations discussed in this dissertation. Most of the CWs are designed for secondary or tertiary treatment, therefore there are not much data about area of wetlands for quaternary treatment in praxis. As we focused in this work on removal of 27 MPs, we could not design the wetlands for removal of each of the compounds. Therefore, the sizes of the wetlands in Reisdorf and Echternach were set fixed, considering that 1 PE will produce 200 liters of wastewater, resulting in specific loads of 60g BOD5 (biochemical oxygen demand -5 days), 120g COD and 11g of TN (total nitrogen) [136]. It is important to say that the achieved removals are given for the CWs of these sizes.

Hydraulic loading rate (HLR)

The HLR is calculated from division of the inflow by the wetland area. Previous studies have proven that the removal efficiency of CWs rises with decreasing HLR [137,138]. It is because higher HLR requires higher number of feeding pulses per day, which is than naturally resulting in lower transfer capacity of oxygen. Furthermore, adsorption of hydrophobic compounds on the substrate decreases due to lower contact time [139]. Examples of different types of CWs, the used operational HLR and targeted pollutants are shown in Tab. 2.3.

Tab. 2.3. Examples of different types of CWs, used HLR and targeted pollutants.

Type of CW	HLR (mm/d)	Type of pollutants	Reference
Surface flow	10	diclofenac, ibuprofen, ketoprofen	[140]
VF	13, 30, 70, 160	carbamazepine, caffeine	[120]
HSSF	28 and 56	carbamazepine, diclofenac, ibuprofen, ketoprofen	[141]
VF	125, 250, 375, 500	COD, TN, TP	[142]

Hydraulic retention time (HRT)

The HRT is calculated from division of the bed volume by the inflow. Naturally, the length of the HRT corresponds to the size of the wetland. In general, it is valid that the longer HRT, the higher are the removal efficiencies of the used systems, which was also demonstrated on examples of removals of various substances, e.g. diclofenac, ketoprofen or clarithromycin [143–145]. The reason, why is increased HRT favoring better removals of the pollutants is, that the removal processes within the CWs (adsorption on the substrate, phytoremediation and bioremedation) are slow processes, therefore, the longer HRT enables completion of these processes [146]. In Tab. 2.4 are shown examples of different types of wetlands, the used operational HRT and targeted MPs.

Tab.2.4. Examples of different types of CWs, used HRT and targeted MPs.

Type of the CW	HRT (h)	Type of MPs	Reference
HSSF	55	ibuprofen, ketoprofen,	[147]
FWS	6	carbamazepine, sulfamethoxazole	[148]
VF	8	carbamazepine, diclofenac, ibuprofen	[149]

2.1.3.1.3 Physicochemical and other design parameters of the wetland

Temperature

It has been reported that higher temperatures (15-25 °C) improve biodegradation processes in the wetlands, especially the ones implementing nitrifying bacteria [150,151]. There have also been reported higher removals of TP under temperatures >15 °C than under temperatures <15 °C [152]. Higher temperatures promote better growth of the plants and their root activity, which results in enhanced phytoremediation. Adsorption is temperature-dependent as well, however favored by low temperature as it is an exothermic process.

pH

The role of pH is crucial especially for the biotic processes and organisms, such as plants and microbes. Therefore, the optimal pH should be around neutral, favoring than plant development and microbial activities [150]. It was proven that low pH is resulting in low removal rates of nitrogen and has toxic effects to wetland plants and microbiological species [153,154] pH also affects dissociation of the MPs and consequent attachment to the substrate via ion exchange [155].

Dissolved oxygen

As mentioned previously, presence of DO in the wetlands is crucial for establishment of the aerobic conditions, which are favoring removal of the pollutants within the CWs thanks to their positive impact on the microbial activity and plant growth (e.g. concentrations of DO >5 mg/l lead to higher oxygen transfer in the wetlands and therefore to higher removals of pollutants [111,156]). This can be demonstrated on example of diclofenac, which was removed better in aerated VFCW (>99%) than in non-aerated VFCW (95%) [157]. Also, the impact of DO on the removal of MPs is discussed in the publication [IV], where the additional aeration of the systems resulted in enhanced global removals of the targeted MPs.

Water quality

CWs are effective technologies for polishing of wastewater effluents before the direct discharge to the water bodies. Besides removal of MPs, they are efficient e.g. for removal of other pollutants, such as nitrogen or phosphorus, which presence can lead to eutrophication and can contaminate groundwater used for potable supply [158]. From the target discharge limit of the effluent can be specified the inflow concentration for the CW, for example, in case of municipal wastewater treatment, if the goal for BOD is 20-30 mg/l, than the concentration of BOD in the influent to the CW should be 50-100 mg/l [111]. As a specific case, for usage of wetlands for the polishing wastewater effluents in Florida, the concentrations of TN (total nitrogen) and TP (total phosphorus) have to be less than 75 $g/m^2 \cdot year$ and 9 $g/m^2 \cdot year$, respectively [111]. The performance of the VFCWs is dependent on the type of the wastewater's pretreatment as well, e.g. on choice of a septic or settlement tank.

2.1.3.1.4 Configuration and operation examples

The VFCW can be operated in different configurations.

General division of the VF wetlands is down flow, up flow and fill and drain while the down flow configuration is the most common one [101,159]. In the down flow configuration is the wastewater delivered intermittently on the surface of the unit allowing aerobic filtration through the bed. The French vertical flow wetland, is a specific down flow configuration, containing two subsequent vertical stages with varying filter media. This specific design allows treatment of raw wastewater after trespassing a basic screen. First stage (for the raw wastewater) is often called French reed bed, followed usually by a VF wetland applied in the second stage. Among advantages of these systems belong e.g. stability against varying hydraulic loadings or lower risk of clogging than in case of HSSF wetlands [160]. In the up flow systems is the wastewater distributed across the bottom of the filter and is consequently moving to the bed surface. These systems are than saturated by water and therefore anoxic or anaerobic conditions are established. However, this configuration is not used very frequently. General illustration of down flow and up flow configurations is shown on Fig. 2.7.

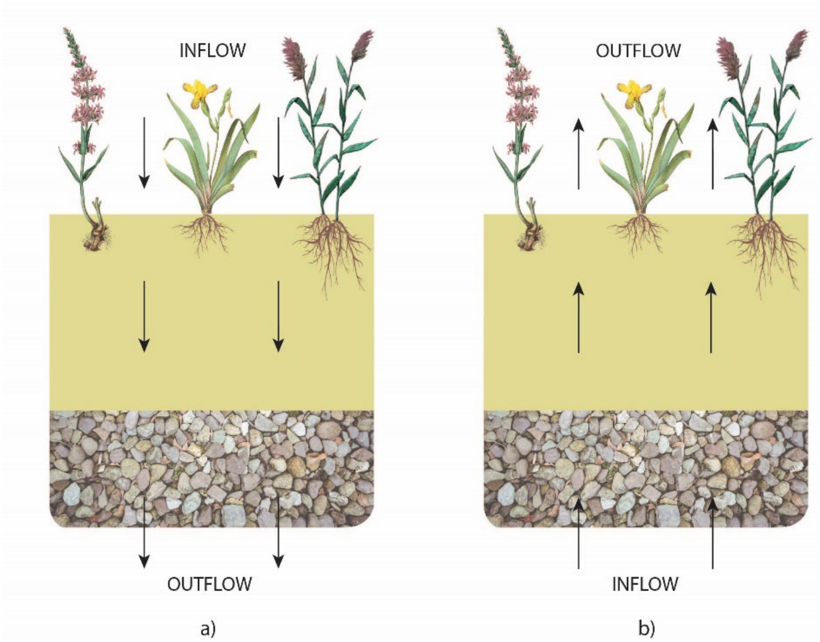


Fig. 2.7. Scheme of a VFCW in down flow configuration (a) and up flow configuration (b).

The fill and drain system is alternating between down and up flow. Advantage of this system is, that thanks to the alternation it is able to provide conditions to remove both ammonia and nitrate [101,161]. The mentioned systems can be combined, for example, one VF unit can be fed with the wastewater vertically downwards and the second one vice versa, from the bottom to the top upwards [162].

The VF wetlands can also be used in series, under the same feeding conditions, but varying e.g. in used plants or types of substrate [163]. This allows to assess efficiencies of the used media and was used also in the cases of the lab and some pilot scale installations of the project targeted in this dissertation. There are some studies about usage of the VF wetlands in parallel configuration too. This configuration leads to enhanced removal of nitrogen or phosphorus [164].

In Europe is often used so-called hybrid configuration of the VFCW with the HSSF, where the VF wetlands are positioned before the horizontal wetlands in order to remove total suspended solids (TSS) and organic compounds which could cause clogging in the HSSF wetlands and additionally the VFCW converts ammonia to nitrate. In the HSSF wetland denitrification takes place and organic compounds are removed simultaneously. The opposite configuration (HSSF before VF) is designed for optimized nitrification (oxidation of ammonia to nitrite and consequently to nitrate) in the VFCW and for removal of TSS and organics in the HSSF wetland. Here, the outflow needs to be recirculated to the inflow and then denitrification takes place in the HSSF part.

Knowledge gaps

The current research about CWs used for removal of MPs has still some knowledge gaps, even though this technology is being used for many decades. Some of these gaps are:

- Effect of long term operation on treatment efficiency of CW [165];
- Optimization of vegetation maintenance [166];
- Hydraulics barriers of surface flow wetlands (targeted in this work) [167];
- Relevant removal mechanisms of MPs in large-scale CWs [168];
- Removal of emergent contaminants (targeted in this work);
- Removal of pathogens [169];
- Contribution of microbial communities to removal of MPs (targeted in this work) [170,171].

- Role of iron and its multi-interactions in processes of CWs (impact on microbiological and chemical transformations during treatment of wastewater in CWs) [172];
- Pollution swapping (increase of one contaminant as a result of a measure introduced to reduce a different contaminant – e.g. if pollutants such as carbon or nitrogen are caught, their transfer within the wetland results to production of the greenhouse gasses (methane, carbon dioxide or nitrous oxide)) [173];

As anticipated, some of the mentioned knowledge gaps (hydraulic barriers, removal of emergent contaminants, and others) are targeted in the PhD research discussed in this dissertation and targeting them is part of the research's motivation.

3 Research significance

3.1.1 Motivation

The involvement of CWs as an AWWT technology in this research has various motivations:

1. The ability of CWs to remove larger number of selected MPs from municipal effluents of small-to-medium sized WWTPs needs to be investigated;
2. The removal efficiencies of the VFCWs under different operational scenarios in lab- and pilot-scale needs to be evaluated;
3. Influence of the present media and components in VFCWs on their removal efficiency needs to be clarified;
4. Role of the mechanisms contributing to removal of the studied MPs within the CWs needs to be evaluated.

Point 4. draws motivations for the main topic the PhD research - quantification of mechanisms contributing to removal of MPs in VFCWs with the specific aims:

1. Identification of the removal mechanisms in VFCWs;

2. Identification of the most efficient media for each mechanism (soil for adsorption, plant for phytoremediation, plant and rhizosphere system for bioremediation);
3. Relation of each mechanism's role in the context of the whole wetland unit;
4. Identification of the most dominant removal mechanism;
5. Relation of each compound's tendency to the removal mechanism.

The removal mechanisms cannot be split as the mutual synergy of these processes is very complex. However, better understanding about them enables the wetland designer to have certain control over the performance by particular choices. For example, proper choice of a substrate with e.g. higher specific surface area would help compounds, for which most dominant removal mechanism is adsorption, to be adsorbed rather than biodegraded. Proper choice of plants, which would meet needed requirements (eco-toxic stability, tolerance against seasonal and matrix changes, etc.) could enhance uptake of compounds, which are not biodegradable and do not tend to be adsorbed on the substrates. Proper choice of feeding cycles could help to create proper aerobic conditions favorable for activity of the microbes and so to enhance bioremediation of biodegradable compounds.

3.1.2 Objectives

3.1.2.1 Constructed wetlands

The PhD research has been part of the Interreg Greater Region project EmiSûre (Développement de stratégies visant à réduire l'introduction de micro-polluants dans les cours d'eau de la zone transfrontalière germano-luxembourgeoise). This project aimed to find strategies to decrease the emissions of MPs in water bodies with the help of advanced wastewater treatment technology. As mentioned earlier, CWs are chosen as a technology suitable for treatment of the commonly present rural areas of this region, with characteristics similar to the catchment of the river Sûre, which is a geographical border between Luxembourg and Germany. As a PhD researcher of University of Luxembourg, the author of this thesis was involved in experimental work of the project: firstly with the lab-scale installation (six lysimeters = lab-scale CWs treating synthetic wastewater (prepared according to a standard recipe so that the final values meet effluent concentrations typical for a small/medium sized WWTP)), on site of the university and then in set-up and experimental work on a pilot scale (three CWs) at the wastewater treatment plant (WWTP) of Reisdorf (4 300 PE). The author was involved partially in the set-up and experimental work on the pilot-scale (two CWs) on WWTP Echternach (20 000 PE). For simplification, scheme of the wetland installations of the EmiSûre project is illustrated on Fig. 3.1.

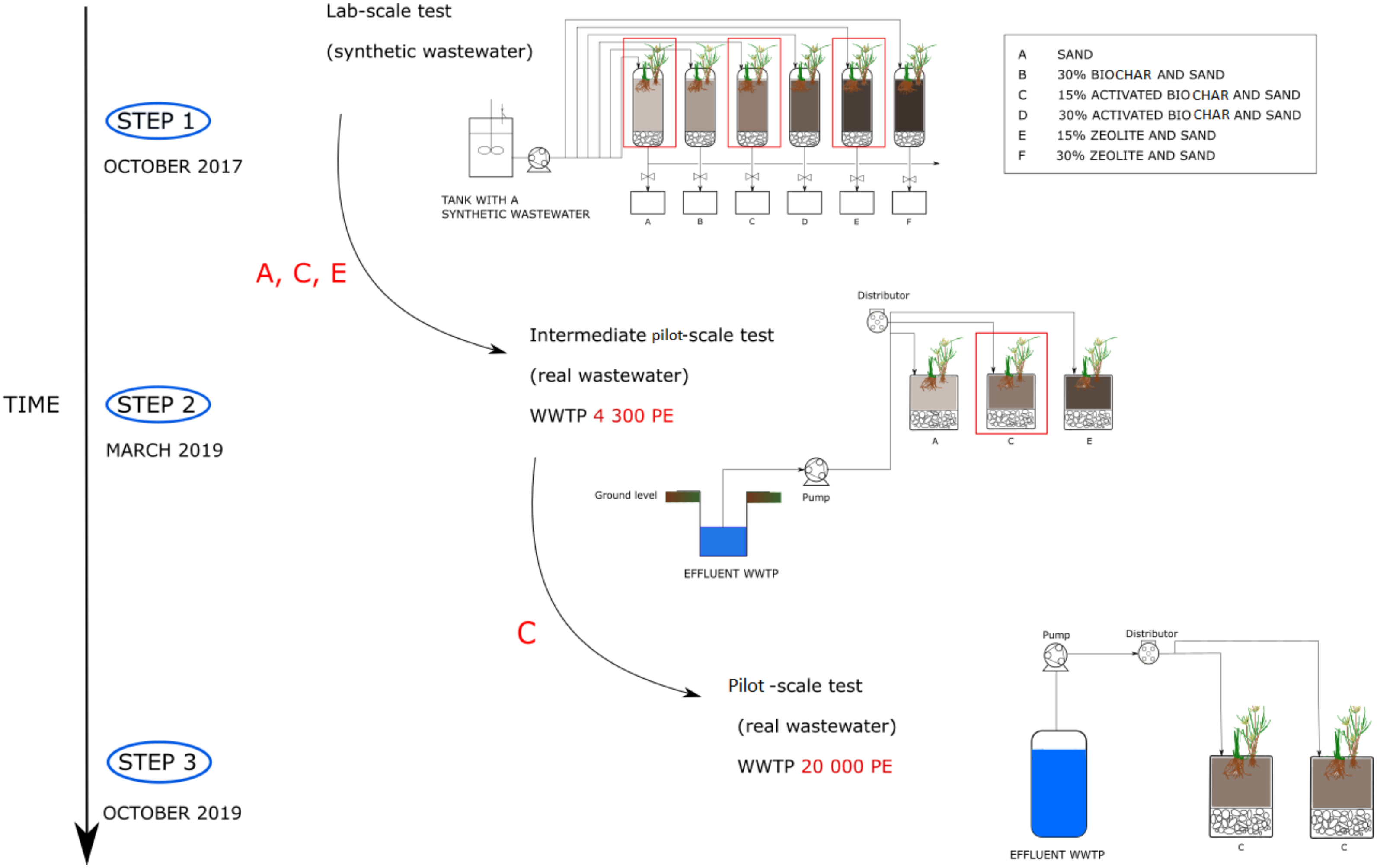


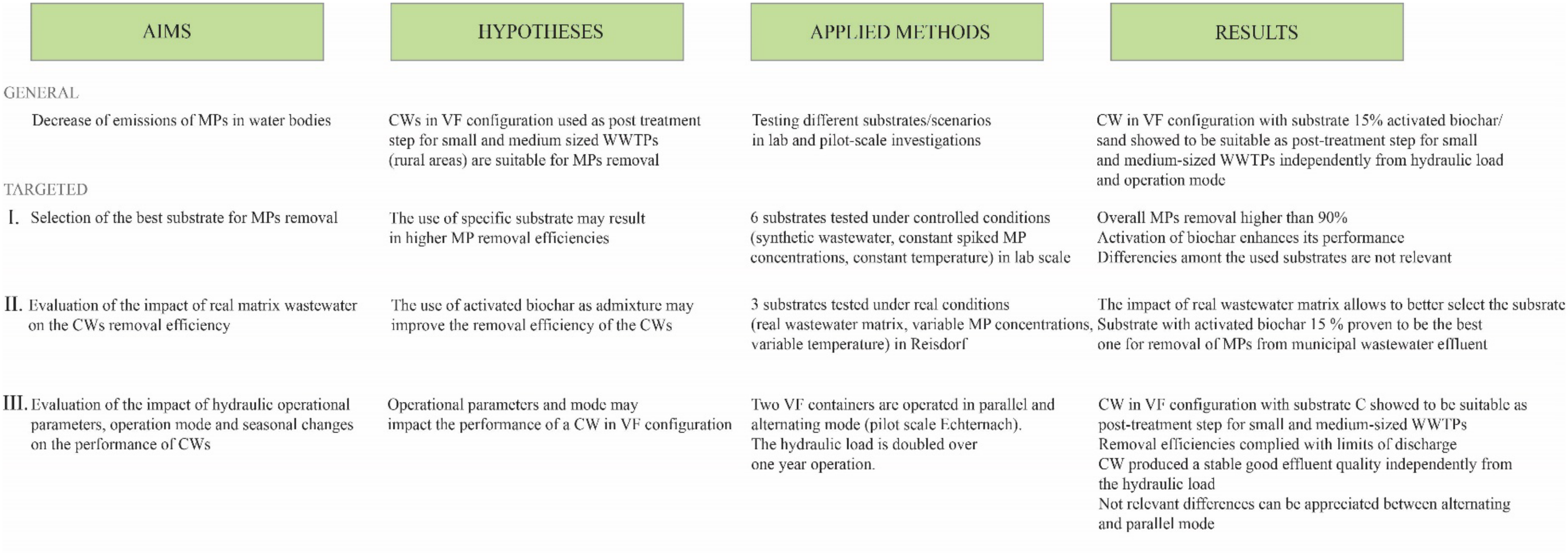
Fig. 3.1. CW experimental scheme Emisûre.

As can be seen from Fig. 3.1., in the laboratory installation were investigated six types of bentonite sand based substrates with admixtures of non- and activated biochar and zeolite. All substrates are fully homogenized. Detailed composition of the lysimeter substrates is shown in Tab. 3.1.

Tab. 3.1.: Detailed composition of the substrate matrix in the lysimeters.

Label	**Composition**
A	sand
B	70 % sand and 30 % non-activated biochar
C	85 % sand and 15 % activated biochar
D	70 % sand and 30 % activated biochar
E	85 % sand and 15 % zeolite
F	70 % sand and 30 % zeolite

For the research investigating CWs have been created aims and hypothesis, which are summarized, together with applied methods and general results for CW installations in Fig. 3.2. The results shown have been achieved within the EmiSûre project and they are considered as a basis of the main PhD research topic.



AIMS	HYPOTHESES	APPLIED METHODS	RESULTS
GENERAL			
Decrease of emissions of MPs in water bodies	CWs in VF configuration used as post treatment step for small and medium sized WWTPs (rural areas) are suitable for MPs removal	Testing different substrates/scenarios in lab and pilot-scale investigations	CW in VF configuration with substrate 15% activated biochar/ sand showed to be suitable as post-treatment step for small and medium-sized WWTPs independently from hydraulic load and operation mode
TARGETED			
I. Selection of the best substrate for MPs removal	The use of specific substrate may result in higher MP removal efficiencies	6 substrates tested under controlled conditions (synthetic wastewater, constant spiked MP concentrations, constant temperature) in lab scale	Overall MPs removal higher than 90% Activation of biochar enhances its performance Differencies amont the used substrates are not relevant
II. Evaluation of the impact of real matrix wastewater on the CWs removal efficiency	The use of activated biochar as admixture may improve the removal efficiency of the CWs	3 substrates tested under real conditions (real wastewater matrix, variable MP concentrations, variable temperature) in Reisdorf	The impact of real wastewater matrix allows to better select the subsrate Substrate with activated biochar 15 % proven to be the best one for removal of MPs from municipal wastewater effluent
III. Evaluation of the impact of hydraulic operational parameters, operation mode and seasonal changes on the performance of CWs	Operational parameters and mode may impact the performance of a CW in VF configuration	Two VF containers are operated in parallel and alternating mode (pilot scale Echternach). The hydraulic load is doubled over one year operation.	CW in VF configuration with substrate C showed to be suitable as post-treatment step for small and medium-sized WWTPs Removal efficiencies complied with limits of discharge CW produced a stable good effluent quality independently from the hydraulic load Not relevant differences can be appreciated between alternating and parallel mode

Fig. 3.2. Overview of aims, hypotheses, applied methods and general results for CW installations.

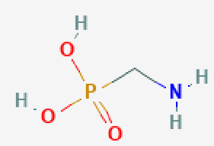
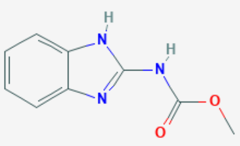
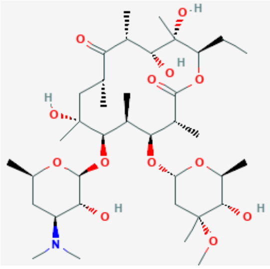
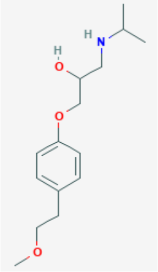
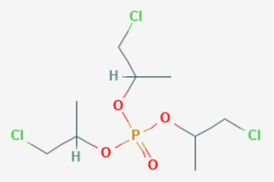
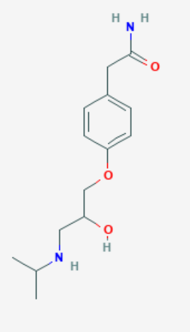
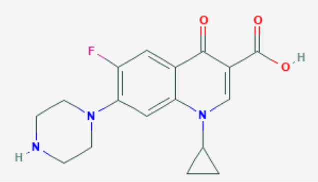
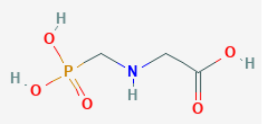
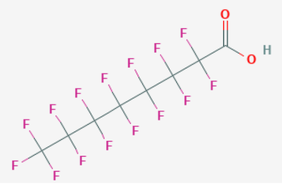
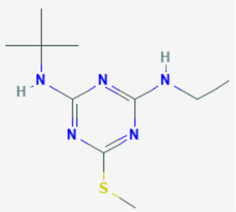
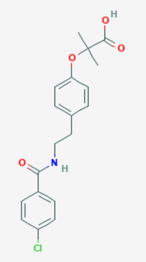
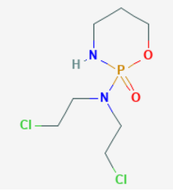
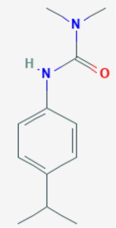
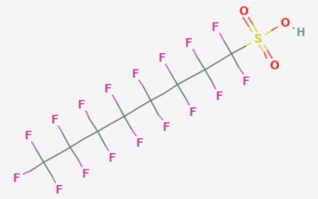
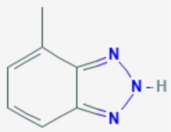
From the Figs 3.1-2. it is clear, that CW with substrate C was the best one for removal of MPs. The studied MPs and their properties are shown in Tabs 3.2 and 3.3.

Tab. 3.2. Selected compounds, their inclusion, EQS, properties and tendencies to removal mechanisms within the CWs.

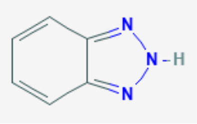
Application	**Compound**	**CAS number**	**Therapeutic Group/Use**	**Selection criteria**	**EQS (µg/l)** [174]	**Log Kow**	**Tendency to a removal mechanism, reference**
Pharmaceuticals and metabolites	Atenolol	29122-68-7	Beta Blocker	Highly prescribed.	150	0.16	Adsorption, [143,175]
	Bezafibrate	41859-67-0	Lipid regulator	Highly biodegradable.	2.3	4.25	Bioremediation, [176]
	Carbamazepine	298-46-4	Psychiatric drug	Mainly excreted as hydroxylated metabolite. Control compound.	2	2.45	Adsorption, [177,178]
	Clarithromycin	81103-11-9	Antibiotic	Present in the Watch List (EU) 2015/495 of 20 March 2015 [179].	0.12	3.16	Adsorption, [180,181]
	Ciprofloxacin	85721-33-1	Antibiotic	Present in the Watch List (EU) 2018/840 [182]	0.089	0.28	Adsorption [183]
	Cyclophosphamide	50-18-0	Cytostatic	High eco-toxicity impact.	N/A	0.63	Phytoremediation, [184]
	Diclofenac	15307-86-5	Analgesic/anti-inflammatories	Present in Directive 2013/39/EU.	0.05	4.51	Bioremediation, adsorption [157,185,186]
	Erythromycin A	114-07-8	Antibiotic	Present in the Watch List (EU) 2015/495 of 20 March 2015 [179].	N/A	8.9	Bioremediation, adsorption, [187,188]
	Ketoprofen	22071-15-4	Analgesic/anti-inflammatories	Highly prescribed. Found in surface waters.	N/A	3.12	Bioremediation, [150,189]
	Lidocaine	137-58-6	Anaesthetic	Highly prescribed. Found in surface waters.	N/A	2.26	Adsorption, [190]
	Metoprolol	51384-51-1	Beta Blocker	Highly prescribed.	8.6	1.88	Bioremediation, adsorption, [188,191]
	Propranolol	525-66-6	Beta Blocker	Highly prescribed. Found in concentrations above EQS.	0.16	3.48	Adsorption, [175]
	N4-acetylsulfamethoxazole	21312-10-7	Metabolite of Sulfamethoxazole	For mass balance.	N/A	0.86	
	Sulfamethoxazole	723-46-6	Antibiotic	Old antibiotic, still in use. Scientific data available.	0.6	0.89	Bioremediation, [192,193]
Pesticides/Herbicides	Carbendazim	10605-21-7	Fungicide	Very persistent.	0.44	1.52	Adsorption, [194]
	DEET	134-62-3	Insect repellent	Very persistent.	88	2.02	Adsorption, [195]
	Diuron	330-54-1	Herbicide	Present in Directive 2018/105/E [182].	0.07	2.68	Adsorption, phytoremediation, [196,197]
	Isoproturon	34123-59-6	Herbicide	Present in Directive 2018/105/E [182].	0.64	2.87	Bioremediation, adsorption, [198,199]
	Terbutryn	886-50-0	Herbicide	Very persistent.	0.065	3.74	Phytoremediation, [200]
	Mecoprop (MCPP)	7085-19-0	Herbicide	Found in surface waters.	3.6	3.13	Adsorption, [201]
	Tolyltriazole	29385-43-1	Fertilizer	Highly used. Most abundant in WWTPs discharging in the Sûre river.	N/A	1.08	Adsorption, [202]
	Glyphosate	1071-83-6	Herbicide	Under discussion.	120	-3.4	Adsorption, [203]
	Aminomethylphosphonic acid (AMPA)	1066-51-9	Degradation product	Under discussion.	1500	-1.63	
Fluorosurfactants	Perfluorooctanesulfonic acid (PFOS)	1763-23-1	Surfactant	Priority compound.	0.002	4.49	Phytoremediation, [204]
	Perfluorooctanoic acid (PFOA)	335-67-1	Surfactant	Of political concern.	N/A	4.81	Phytoremediation, [204]
Corrosion inhibitor	Benzotriazole	95-14-7	Corrosion inhibitor/Antiviral	Highly used. Most abundant in WWTPs discharging in the Sûre river.	240	1.44	Adsorption,[202]
Flame retardant	Tris(2-chloroisopropyl)phosphate (TCPP)	13674-84-5	Flame retardant	Highly used. Most abundant in WWTPs discharging in the Sûre river.	N/A	2.59	Bioremediation, [205]

In the following table are shown chemical structures of all studied compounds.

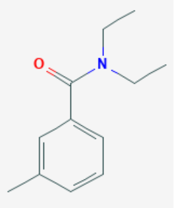
Tab. 3.3. The studied 27 MPs and their chemical structure.

AMPA	Carbendazim	Erythromycin	Metoprolol	TCIPP
				
Atenolol	Ciprofloxacin	Glyphosate	PFOA	Terbutryn
				
Bezafibrate	Cyclophosphamide	Isoproturon	PFOS	Tolyltriazole
				

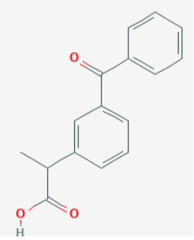
Benzotriazole



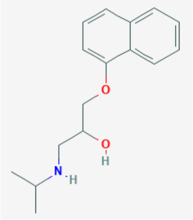
DEET



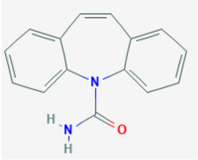
Ketoprofen



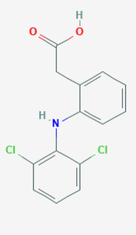
Propranolol



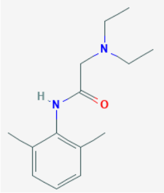
Carbamazepine



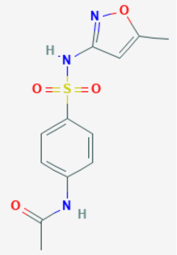
Diclofenac



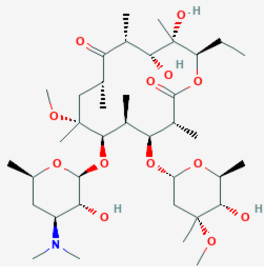
Lidocaine



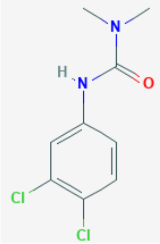
N-Acetyl-Sulfamethoxazole



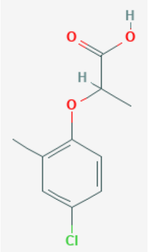
Clarithromycin



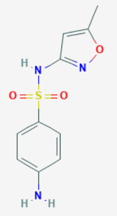
Diuron



MCPP



Sulfamethoxazole



The MPs are chosen by the project research board based on the following aspects, which are briefly shown in the Tab. 3.2.:

1. Compounds known to be relevant in the region (catchment of the Sûre river) and monitored in previous projects (benzotriazole, tolyltriazole, TCPP) [206].
2. Compounds being under legal obligations (isoproturon or diuron) or compounds being under observation (macrolide antibiotics).
3. Compounds with high eco-toxicity (cyclophosphamide) [207].
4. Compounds generally excreted in high amounts (majority of pharmaceuticals (beta-blockers, antibiotics, analgesics, et al.).

In the Tab. 3.2. are shown values of the parameter log Kow, which represents tendency of each compound to different environments (either aquatic or organic). Compounds with higher log Kow tend to be more adsorbed to organic matter and less to water. Compounds with very high log Kow (>4.5) may have bio-accumulating potential in living organisms [208].

Based on the successful results from the application of the CW for removal of MPs when applied as an advanced wastewater treatment step an aim has been developed to quantify the mechanisms, which contribute on the removal of the MPs. The aims and hypotheses of the removal mechanisms are discussed in the next sub-chapter.

3.1.2.2 Quantification of removal mechanisms

As mentioned earlier, the general hypothesis is that the mechanisms contributing to the removal of MPs from wastewater effluents are: adsorption, phyto- and bioremediation. Photoremediation is considered as negligible as the surface of vertical subsurface CWs exposed to sunlight is small [114,209]. When looking at the table 3.2. and 3.3., some tendencies of the studied compounds to the mentioned removal mechanisms can be drawn, such as:

- Beta-blockers tend to be adsorbed to organic substrates, thanks to the π-π interactions between the aromatic circles of the substrate and the compounds.

- Fluorosurfactants are generally very persistent compounds (not well biodegradable) and tend to successive desorption after being adsorbed on the substrate. However, there were recorded some successful attempts for their removal by phytoremediation.
- Carbendazim, DEET, diclofenac, lidocaine and terbutryn are very persistent (not well biodegradable), and their log Kow is higher than 0, therefore they are not very hydrophobic – adsorption on organic substrates seems to be their main removal mechanism.

Based on these general hypotheses different set-ups are designed, which are then applied to achieve elimination of the MPs from the water fraction by experiments simulating each mechanism. For each mechanism is dedicated one publication, the publications create the core of this dissertation. For illustration, the interconnectedness of the mechanisms in the CW and location of the publication containing the theoretical backgrounds, targeted experimental solutions and results of the research shown in Fig. 3.3. The targeted aims and hypotheses considering the mechanisms are shown in Fig. 3.4.

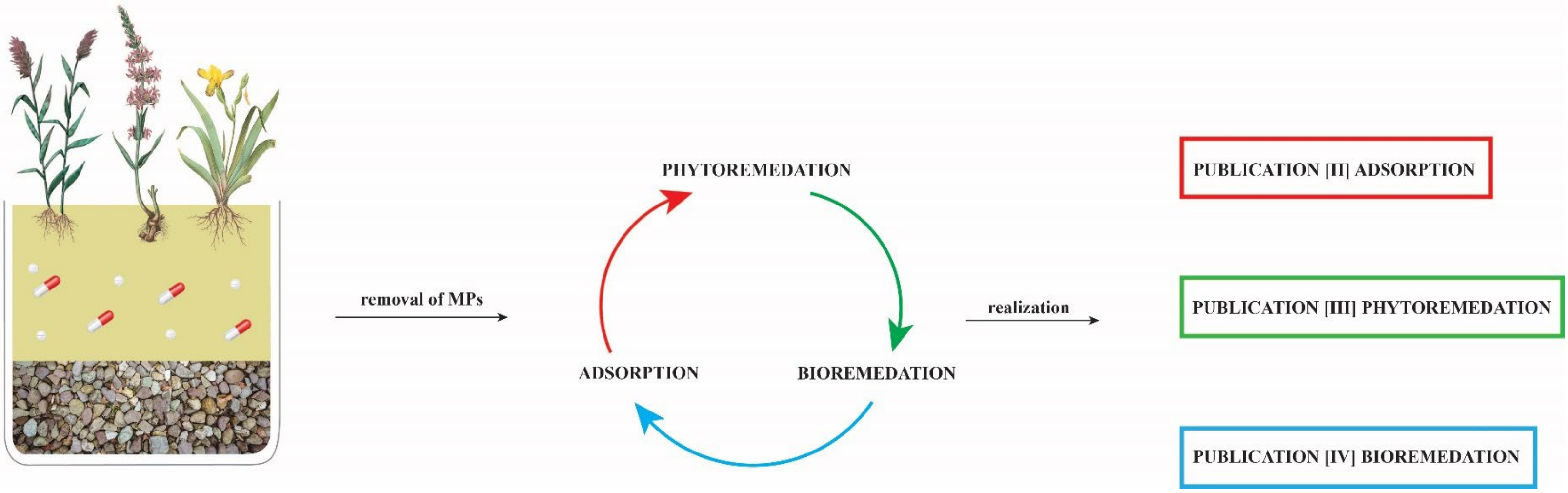


Fig. 3.3. The general hypothesis about removal mechanisms of MPs in CWs and location of the publication discussing targeted issues.

AIMS	HYPOTHESES	APPLIED METHODS
GENERAL		
I. establishment of the contribution of each mechanism for removal of MPs	application of specific substrate or plant may result in higher removals	specific adapted methods
II. determination of the most significant mechanism for removal of MPs		specific adapted methods
TARGETED		
ADSORPTION		
I. establishment of the general adsorption efficiency of the three most effective substrates	mixture of bentonite sand with 15% activated biochar might show highest adsorption ability	adsorption kinetic with dye as proxy
II. determination of the adsorption efficiency for removal of MPs of the three most effective substrates	mixture of bentonite sand with 15% activated biochar might show highest adsorption ability	packed-bed columns with mixture of 27 MPs
PHYTOREMEDIATION		
I. establishment of the up take potential of the plants for MPs	some plants may show better removal efficiency (*Lythrum salicaria* is able to create extensive root system with established AMF symbiosis, which is beneficial for phytoremediation)	experiments in hydroponic conditions with the studied types of plants and mixture of 27 MPs
BIOREMEDATION		
I. establishment of the contribution of the rhizosphere microbiome to the removal of MPs	certain bacterial genera might have a direct impact for the removal of MPs	bioremediation experiments in semi-hydroponic conditions
II. identification and abundance of the present bacterial genera		16S rRNA amplicon sequencing method
III. determination of the colonization by the arbuscular mycorrhizal fungi of the plant roots	the fungi create symbiotic relationship with the plants resulting in higher removal of MPs	microscopical observation & grid-line intersect method

Fig. 3.4. The targeted hypotheses about removal mechanisms of MPs in CWs and subsequent experimental designs.

4 Thesis structure

The research of the dissertation is presented in the first chapter called General introduction, followed by State of the art and Research significance. The main part of the thesis is divided into four main domains in line with the PhD research objectives. These domains are:

1. Publication [I]: CW installations: Set-up of the installation (CW Reisdorf, 4 300 PE), experimental, analytical work on lab- and pilot-scale wetland installations (CW Reisdorf, 4 300 PE), treatment of the data and evaluation of the results. For the better understanding of processes in the wetlands contributing on the removal of the MPs, an aim has been established to quantify these processes, which are namely: adsorption, phytoremediation and bioremediation.
2. Publication [II]: Removal mechanisms – adsorption: To evaluate the potential of the three most efficient soil substrates from the CW installations to adsorb the studied MPs, two types of experiments have been designed. In the first procedure, adsorption kinetics with a single compound (dye as proxy) have been performed. These tests allow to gain the perception about the adsorption capacity of studied substrates compared to conventional substrates (granular activated carbons). For the second procedure has been established an installation with three packed-bed columns filled with the studied substrates and a mixture of 27 studied MPs have been fed through the columns. Results of these two experiments describe the adsorption properties of the studied substrates for removal of MPs and help to establish the role of adsorption in the wetland removal processes.
3. Publication [III]: Removal mechanisms – phytoremediation: To evaluate the potential of the plants to up take studied MPs from water, an experiment in hydroponic conditions has been designed. Three types of emergent macrophytes used in the previous CW installations are exposed to mixture of the 27 studied MPs. In order to evaluate pure contribution of the plants, the roots of the plants are disposed of the rhizosphere soil and microbiome. After the experiment, the ability of the plants to up take MPs is evaluated and the role of phytoremediation in the wetland removal processes is established.

4. Publication [IV]: Removal mechanisms – bioremediation: To evaluate the potential of present rhizosphere microbiome for the removal of the MPs, a tailored experimental set-up in semi-hydroponic conditions has been developed. Three types of emergent macrophytes used in the previous CW installations with preserved rhizosphere microbiome are exposed to mixture of the 27 studied MPs. Due to previously carried phytoremediation experiments it is possible to establish the potential of pure plants. Having this information as a start, is additionally possible to determine the contribution of the rhizosphere microbiome to the removal of MPs.

The major outcomes of the performed research represented in publications I-IV and insights for future research are represented in the Closing chapters.

4.1 Presentations of the publications and links between them

This sub-chapter is devoted to introduction and links between each research paper of this cumulative dissertation. The research papers are ordered based on the PhD research sequence, not based on the date of publication. The author of this dissertation is first author of publications [II – IV] and co-author of publication [I].

4.1.1 Publication I

Reference [I] - Venditti, S., Brunhoferova, H., & Hansen, J. (2022). Behaviour of 27 selected emerging contaminants in vertical flow constructed wetlands as post-treatment for municipal wastewater. *Science of The Total Environment*, 819, 153234. https://doi.org/10.1016/J.SCITOTENV.2022.153234

Short abstract

In the first publication are introduced and investigated six unconventional sand-based substrates (sand enriched with activated or non-activated biochar or zeolite in different ratios) in VFCWs planted with *Phragmites australis* and *Iris pseudacorus* for removal of the studied 27 MPs. The results of laboratory investigations show, that the overall removal efficiencies exceed 90% for most of the cases. Three of the most effective substrates from laboratory investigations were tested under real conditions, resulting in VFCW with 15% activated biochar as an admixture to betonite sand to be the most effective substrate for removal of the studied MPs.

Keywords: micropollutants, municipal wastewater effluent, post-treatment, vertical flow constructed wetlands, removal mechanisms.

Journal metrics and citations

- Status: Published in January 2022 in Science of the Total Environment, Elsevier (Netherlands)
- Q1 rating in Environmental Chemistry (SJR Journal Ranking)
- Current impact factor (IF): 7.963 (2022)
- Citations: 2 (Google Scholar)

4.1.2 Publication II

Reference [II] - Brunhoferova, H., Venditti, S., & Hansen, J. (2022) Characterization of unconventional sand based substrates for adsorption of micropollutants in nature-based systems. *Journal of Environmental Management*, 318, 115593. https://doi.org/10.1016/j.jenvman.2022.115593

Short abstract

The focus of the second publication is given on characterization of the three most effective substrates investigated in publication [I] (bentonite sand, sand with 15 % activated biochar and sand with 15 % zeolite). For that is chosen a two-side path: adsorption kinetics with a single compound (dye as proxy) and adsorption on packed-bed columns of the studied 27 MPs. Additionally, all the three substrates are characterized for their physical and chemical properties. The most effective substrate for removal of MPs is 15% activated biochar as an admixture to betonite sand, resulting in removal of 18 out of 27 compounds with high efficiency (80 – 99%). To complete the investigation about adsorption's role in removal of MPs, adsorption models for all compounds are created, resulting in the combined Langmuir-Freundlich model to be the best fit for the experimental data.

Keywords: unconventional substrates, adsorption processes, micropollutant removal, isotherm models.

Journal metrics and citations

- Status: Published in June 2022 in Journal of Environmental Management, Elsevier

(Netherlands)

- Q1 rating in Environmental Engineering (SJR Journal Ranking)
- Current impact factor (IF): 6.789 (2022)
- Citations: 0

4.1.3 Publication III

Reference [III] - Brunhoferova, H., Venditti, S., Schlienz, M., & Hansen, J. (2021). Removal of 27 micropollutants by selected wetland macrophytes in hydroponic conditions. *Chemosphere*, 281, 130980. https://doi.org/10.1016/j.chemosphere.2021.130980

Short abstract

Third publication addresses up take potential of the macrophytes used in the EmiSûre wetland installations (*Phragmites australis, Iris pseudacorus* and *Lythrum salicaria*) of the studied 27 MPs. The experiments are carried out in hydroponic conditions and plants in different vegetative status are compared. The plant with the highest ability to remove MPs is *Lythrum salicaria*, which was able to remove 25 out of 27 compounds with more than 20% efficiency.

Keywords: micropollutant removal, emergent macrophytes, hydroponic conditions.

Journal metrics and citations

- Status: Published in May 2021 in Chemosphere, Elsevier, (United Kingdom)
- Q1 rating in Chemistry (miscallenous) (SJR Journal Ranking)
- Current impact factor (IF): 7.086 (2022)
- Citations: 10 (Google Scholar)

4.1.4 Publication IV

Reference [IV] - Brunhoferova, H.; Venditti, S.; Laczny, C.C.; Lebrun, L.; Hansen, J. (2022) Bioremediation of 27 Micropollutants by Symbiotic Microorganisms of Wetland Macrophytes. *Sustainability*, 14, 3944. https://doi.org/10.3390/su14073944

Short abstract

The last mechanism, which is to be described, is bioremediation, which role in removal of MPs is investigated in the fourth publication. To address the role of the rhizosphere microbiome were created 3 systems in semi-hydroponic conditions consisting of the studied plants (*Phragmites australis, Iris pseudacorus* and *Lythrum salicaria*) and the rhizosphere microbiome in the root area soil. As a result of these experiments was found out, that *Iris pseudacorus* is the most effective system, being able to remove 22 out of 27 compounds with more than 80% efficiency. To include complete information about bioremediation, the present bacterial genera are identified and quantified and genera with ability to remove MPs are specified (*Pseudomonas, Flavobacterium, Variovorax, Methylotenera, Reyranella, Amaricoccus* and *Hydrogenophaga*). Furthermore, colonization of the plant roots by AMF, which create symbiotic relations with the plant roots resulting in enhanced MPs' phytoremediation, is determined (system exhibing highest colonization was *Iris pseudacorus* (56%).

Keywords: arbuscular mycorrhizal fungi, bioremediation, constructed wetlands, removal of micropollutants, rhizosphere microbiome.

Journal metrics and citations

- Status: Published in March 2022 in Sustainability, MDPI, (Switzerland)
- Q1 rating in Geography, Planning and Development (SJR Journal Ranking)
- Current impact factor (IF): 3.889 (2022)
- Citations: 1 (ResearchGate)

4.2 Coherence between each paper

Introduction to the problematics, experimental set-ups of the CW installations, methodology and results is discussed in detail in the publication [I]. As a result of the research described in publication [I] it was found out, that overall removal efficiencies of most of the compounds exceed 90 % of the global removals. Based on the successful application of the CWs as an advanced treatment step for municipal wastewater effluents and aim has been developed to quantify the mechanisms contributing on the removal of MPs by the CWs. Some of the separate mechanisms do not relate to the others – it is a case of adsorption (described in detail in publication [II]). Results of the adsorption experiments do not have any influence on the performance or results of the other experiments. Also, adsorption experiments have been performed before the bioremediation experiments, which in contrary are dependent on the phytoremediation experiments. Based on these reasons and therefore for the coherence of the dissertation, the first removal mechanism discussed is adsorption - publication [II]. Following adsorption comes phytoremediation – publication [III]. Design of these experiments is based on available literature background and it is decided to perform the phytoremediation experiments in hydroponic conditions. These experiments allow to estimate the potential of the plain plants (without rhizosphere microbiome) to remove MPs from the water. With this knowledge, it is possible to design bioremediation experiments, described in publication [IV]. The design itself does not originate from a literature background; it is a result of the observation of the used methods (phytoremediation) and efforts for obtainment of the knowledge of contribution of the rhizosphere microbiome. Classical methods, such as inoculation by the bacteria could have been used. However, this method would have certain insufficiencies, such as:

- composition of the inoculated microbiome would not be identical to the one present naturally in the studied CW;
- abundance of the microbiome naturally present in the studied CW would differ as well, as the wetland was operated under diverting conditions (temperature differences, fluctuations of inlet concentrations of nutrients and MPs).

This could also result into different removals of the MPs in contrary to a wetland with naturally developed microbiome.

5 Publication I - Behaviour of 27 selected emerging contaminants in Vertical Flow Constructed Wetlands as post-treatment for municipal wastewater

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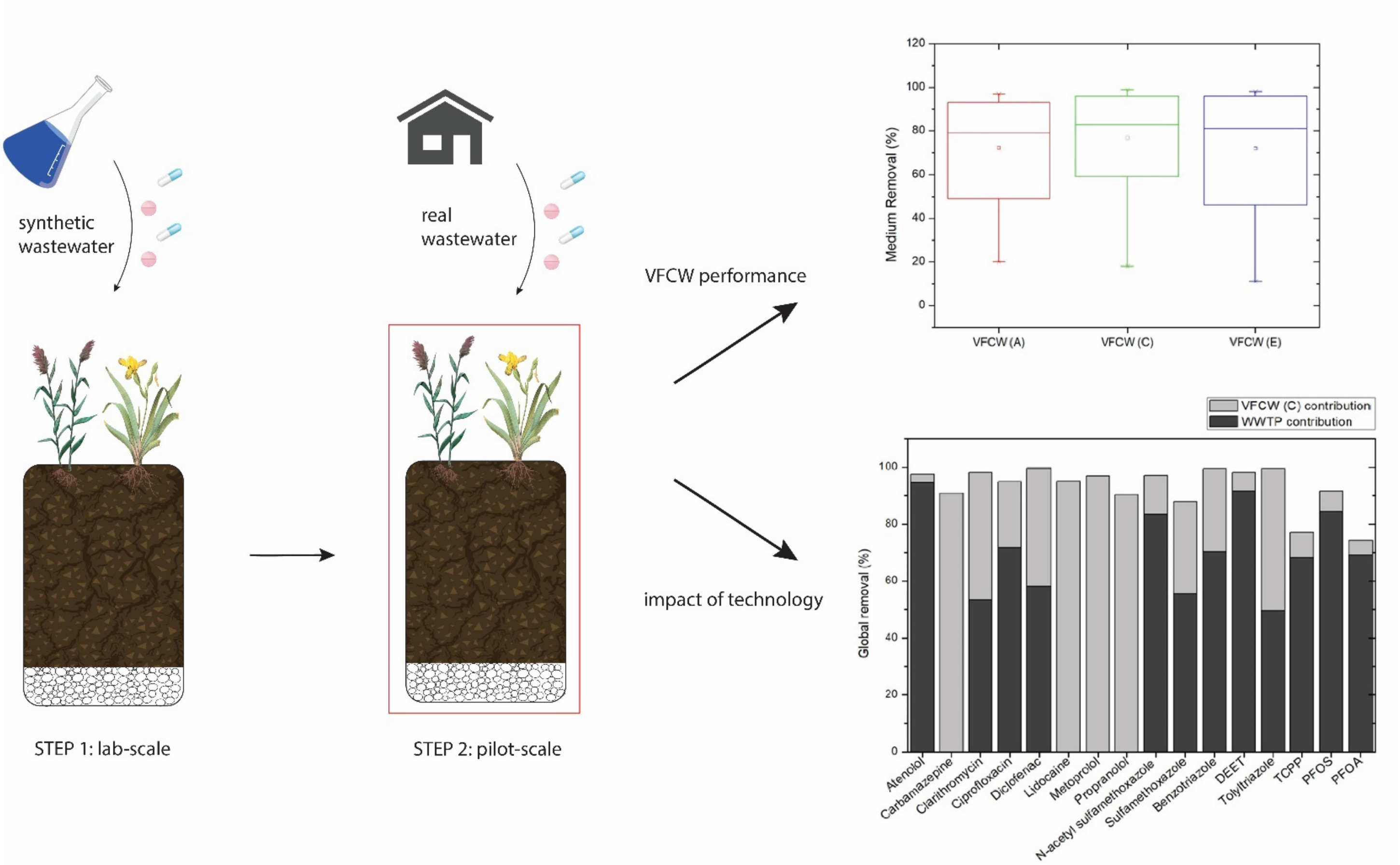
Graphical abstract



Highlights:

- Unconventional substrates are suitable in wetlands for micropollutants elimination.
- The use of activated biochar versus sand/zeolite resulted in a higher efficiency.
- Persistent compounds (i.e. carbamazepine) are well eliminated (>90%).

- The amount of contaminant adsorbed per gram of substrate is quantified.
- Vertical Flow Constructed Wetlands is a promising post-treatment step.

Abstract

Six substrates (i.e. sand enriched with activated or non-activated biochar or zeolite in different ratios) were tested in Vertical Flow Constructed Wetlands (VFCWs) planted with *Phragmites australis* and *Iris pseudacorus* for the removal of 27 emerging contaminants from municipal wastewater. The laboratory investigation under controlled conditions (spiked constant concentrations in synthetic wastewater) lasted 357 days and proved VFCWs being able to provide excellent effluent quality in terms of both macro - and micropollutant elimination. Because overall removal efficiencies exceeded 90 % in most of the cases, significant differences among the substrates were not detectable. For compounds with medium elimination (i.e. AMPA) the type of substrate seemed to play a strong role and the maximum amount of active ingredient adsorbed per amount of substrate has been quantified (i.e. 0.77 ug of AMPA per g of 30 % biochar mixed with sand). Three of the most promising substrates from laboratory where thus selected to be tested under real conditions (fluctuation in concentration, variable temperature). As result, VFCWs with 15 % activated biochar mixed with sand proved to be effective in the removal of 18 emerging contaminants and complying with national discharge standards for 4 selected compounds.

Keywords: micropollutants, municipal wastewater effluent, post-treatment, vertical flow constructed wetlands, removal mechanisms

5.1 Introduction

The occurrence of emerging contaminants (also known as micropollutants, MPs) in surface water has been increasing over the last decades resulting in potential risk for aquatic and terrestrial ecosystems [210]. Hence, the European Commission (EC) decided to identify those substances that could delay or hamper the achievement of a *good ecological and chemical status* for both ground and surface waters as main principle of the Water Framework Directive (2000/60/EC) to be reached by 2027 [211]. With the so-called Watch List [182,212] , the Commission Implementing Decision made it finally mandatory for all EU member states to monitor 17 compounds, among them the antibiotics azithromycin, clarithromycin and erythromycin.

Because of their anthropogenic origin, emerging contaminants are mainly released by Wastewater Treatment Plants (WWTPs) which demonstrated to be inefficient [2] in the removal of the most persistent. Consequently, some countries have set up more strict national discharge standards, started to plan and implement additional treatment steps identifying urban areas which are significant point sources in the river basin they are located in. As example, the Swiss government identified 100 out of the 700 national WWTPs that will be supported with a post-treatment in order to achieve an overall average of 80 % removal of selected organic compounds [213]. Likewise, Germany progressed upgrading 30 WWTPs for MPs elimination [214] with the regions of North Rhine-Westphalia and Baden-Wuerttemberg [215] as front runners. Following this example, Luxembourg released a guideline [216] for those WWTPs that have been selected to be upgraded with a post-treatment. The effluent quality has to achieve 80 % removal for each compound namely diclofenac, carbamazepine, clarithromycin and benzotriazole with the option of adding to the permit substances that are relevant for the respective WWTP when measured above Environmental Quality Standards (EQSs) in the receiving surface waters. Till now, the majority applied common technologies such as Activated Carbon Filtration (both powdered, PAC and granulated, GAC) and Ozone.

However, as the upgrading of WWTPs for MPs elimination is a long-term investment that would affect additional energy consumption of about 30 % [217], some regional studies tried to identify suitable alternative especially for small and medium-sized WWTPs (i.e. less than 20 000 PE). Due to their low investment and operation cost combined with good effluent quality, Constructed Wetlands (CWs) are a promising additional step for micropollutants removal.

CWs are nature-based wastewater treatment processes in which the synergy of multiple mechanisms acting simultaneously (i.e. sorption, photodegradation, phytodegradation, and biodegradation) demonstrated to enhance the removal of heavy metals, organics and nutrients [218][219]. When applied as decentralized treatment for hospital/domestic wastewater and grey water, CWs showed satisfying removal of pharmaceuticals and personal care products [119,157,220] but very little is known about their application as post-treatment. Comprehensive reviews [102,138,221] collected and processed a large number of data highlighting high variability in removal rate values with significant standard deviations.

A better understanding of the key factors influencing the removal of MPs in a CW environment would help to select the appropriate configuration (i.e. surface and subsurface flow, vertical and horizontal) and eventually its operating parameters (i.e. aerobic/anaerobic conditions, intermittent or alternating operation mode, hydraulic retention time and so on). Whether such configuration could impact the removal of individual MP is however associated with the nature of the MP itself.

Because of their hydrophobic nature or electrostatic reactions, some MPs would have a better tendency to be attached on the soil [222] and thus the selection of the filling material would determine the efficiency of the configuration towards those compounds. Sorption may occur via absorption (hydrophobic interactions characterized by the octanol-water partition factor, Log*Kow*) and adsorption (electrostatic interactions characterized by the dissociation constant, p*Ka*): compounds with high Log*Kow* (hydrophobic) have intuitively more affinity for the solid fraction [223]. This is the case of the musk fragrance tonalide [157] removed up to 80 %, the antibiotic class of fluoroquinolones, namely ciprofloxacin and ofloxacin [224] and macrolides, namely azithromycin and clarithromycin [119].

Then, as CWs are exposed to sunlight photochemical reactions are expected to be relevant for those compounds considered photosensitive like fluoroquinolones [225] thus seasonal variation could strongly influence their removal [100,119,188,226]. Photodegradation is however negligible with subsurface flow CW especially in vertical configuration where the surface exposed to sunlight is smaller. Photolysis of diclofenac has also been previously observed [227] and seemed to be more dominant in unplanted wetlands with subsurface flow configuration.

If planted, CWs can act via plant uptake of MPs depending on type of plant [228], plant density and plant root amounts. The role of plant uptake is generally known to be combined with those of biodegradation and strongly affected by seasonal variation [150,229,230]. The presence of microbial community structures near the plant roots (rhizosphere) in co-habitat with Mycorrhiza fungi could enhance the whole biodegradation mechanism. For emerging contaminants which are persistent and not easily biodegradable, the configuration of CWs and the operation conditions may play a great role in their degradation while for contaminants already biodegradable the impact will be minimal.

Without doubt, the complexity of all simultaneous mechanisms and the limited number of studies on these topics has been detrimental in advancing knowledge and trust in the feasibility of CWs for removal of emerging contaminants.

In this context, the Interreg Greater Region project EmiSûre (Développement de stratégies visant à réduire l'introduction de micro-polluants dans les cours d'eau de la zone transfrontalière germano-luxembourgeoise) aimed to exploit the potential of CWs for the mitigation of MPs in rural areas with characteristics similar to the Sûre catchment, a river on the border between Luxembourg and Germany. The main objectives of this study are thus: (1) to conduct a robust investigation on 27 representative emerging contaminants using laboratory tests under controlled conditions (2) to draw conclusions about possible (and most dominant) removal mechanisms with respect to the individual compound (3) to validate and prove CWs in Vertical subsurface Flow (VF) configuration as polishing treatment for small and medium sized wastewater treatment effluents.

5.2 Materials and methods

5.2.1 Selection of target compounds

The comprehensive list of compounds monitored in this study is the result of a joined agreement between the partners of the Interreg Greater Region project 'EmiSûre', among them decision makers (Administration de gestion de l'eau in Luxembourg, Ministerium für Umwelt, Landwirtschaft, Ernährung,Weinbau und Forsten in Rheinland-Pfalz in Germany), WWTP operators (Syndicat Intercommunal de Dépollution des Eaux résiduaires du Nord SIDEN and de l'Est SIDEST both in Luxembourg, and Entsorgungsverband Saar in Germany) and scientists (University of Luxembourg and University of Kaiserslautern in Germany). The 27 compounds (Table 5.1) were selected taking into account: a) those known to be excreted in the highest amount (in the case of pharmaceuticals: antibiotics, beta-blockers, analgesics etc), b) those known with the highest eco-toxicity (i.e. cytostatics), c) those known to be under observation (i.e. macrolides) or with legal obligations (i.e. isoproturon, diuron), d) those known to be especially relevant for the Sûre river and already monitored in previous projects (i.e. benzotriazole, tolyltriazole and tris(2-chloroisopropyl)phosphate) [231].

Table 5.1 : List of compounds

Application	Compound	CAS number	Therapeutic Group/Use	AA-EQS* Chronic quality standard (ug L^{-1})	Spiked concentrations (ug L^{-1})***	Selection criteria
Pharmaceuticals and metabolites	Atenolol	29122-68-7	Beta Blocker	150	5	Highly prescribed.
	Bezafibrate	41859-67-0	Lipid regulator	2.3	5	Highly biodegradable.
	Carbamazepine	298-46-4	Psychiatric drug	2	2	Mainly excreted as hydroxylated metabolite. Control compound.
	Clarithromycin	81103-11-9	Antibiotic	0.12	5	Present in the Watch List (EU) 2015/495 of 20 March 2015 [179].
	Ciprofloxacin	85721-33-1	Antibiotic	0.089	5	Present in the Watch List (EU) 2018/840 [182]
	Cyclophosphamide	50-18-0	Cytostatic	NA**	5	High eco-toxicity impact.
	Diclofenac	15307-86-5	Analgesic/anti-inflammatories	0.05	5	Present in Directive 2013/39/EU.
	Erythromycin A	114-07-8	Antibiotic	NA	5	Present in the Watch List (EU) 2015/495 of 20 March 2015 [179].
	Ketoprofen	22071-15-4	Analgesic/anti-inflammatories	NA	5	Highly prescribed. Found in surface waters.
	Lidocaine	137-58-6	Anaesthetic	NA	5	Highly prescribed. Found in surface waters.
	Metoprolol	51384-51-1	Beta Blocker	8.6	5	Highly prescribed.
	Propranolol	525-66-6	Beta Blocker	0.16	5	Highly prescribed. Found in concentrations above EQS.
	N4-acetylsulfamethoxazole	21312-10-7	Metabolite of Sulfamethoxazole	NA	2	For mass balance.
	Sulfamethoxazole	723-46-6	Antibiotic	0.6	2	Old antibiotic, still in use. Scientific data available.
Pesticides/Herbicides	Carbendazim	10605-21-7	Fungicide	0.44	1	Very persistent.
	DEET	134-62-3	Insect repellent	88	2	Very persistent.
	Diuron	330-54-1	Herbicide	0.07	1	Present in Directive 2018/105/E [182].
	Isoproturon	34123-59-6	Herbicide	0.64	1	Present in Directive 2018/105/E [182].
	Terbutryn	886-50-0	Herbicide	0.065	2	Very persistent.
	Mecoprop (MCPP)	7085-19-0	Herbicide	3.6	1	Found in surface waters.
	Tolyltriazole	29385-43-1	Fertilizer	NA	2	Highly used. Most abundant in WWTPs discharging in the Sûre river.
	Glyphosate	1071-83-6	Herbicide	120	5	Under discussion.
	Aminomethylphosphonic acid (AMPA)	1066-51-9	Degradation product	1500	5	Under discussion.
Fluorosurfactants	Perfluorooctanesulfonic acid (PFOS)	1763-23-1	Surfactant	0.002	2.5	Priority compound.
	Perfluorooctanoic acid (PFOA)	335-67-1	Surfactant	NA	2.5	Of political concern.
Corrosion inhibitor	Benzotriazole	95-14-7	Corrosion inhibitor/Antiviral	240	5	Highly used. Most abundant in WWTPs discharging in the Sûre river.
Flame retardant	Tris(2-chloroisopropyl)phosphate (TCPP)	13674-84-5	Flame retardant	NA	5	Highly used. Most abundant in WWTPs discharging in the Sûre river.

*https://www.ecotoxcentre.ch/expert-service/quality-standards/proposals-for-acute-and-chronic-quality-standards/

**NA: ‘’not available’’ in the Ecotoxcentre database

***spike in the synthetic wastewater prepared for the lab-scale wetlands of this study

5.2.2 Experimental design

5.2.2.1 Lab-scale wetlands set up

The vertical-flow lab scale-wetlands (lysimeters) consist of six plexiglass columns (Europlex, Belgium) having same dimensions (inner diameter 29 cm, height 115 cm) and set up to investigate the impact of a substrate in the removal of MPs from synthetic wastewater used as influent.

The columns are successively filled, from the bottom to the top, with a 10 cm layer of gravel acting as drainage (5 cm of coarse 4-8 mm and 5 cm of fine 2-8 mm), a 90 cm of packing substrate (Figure 5.1).

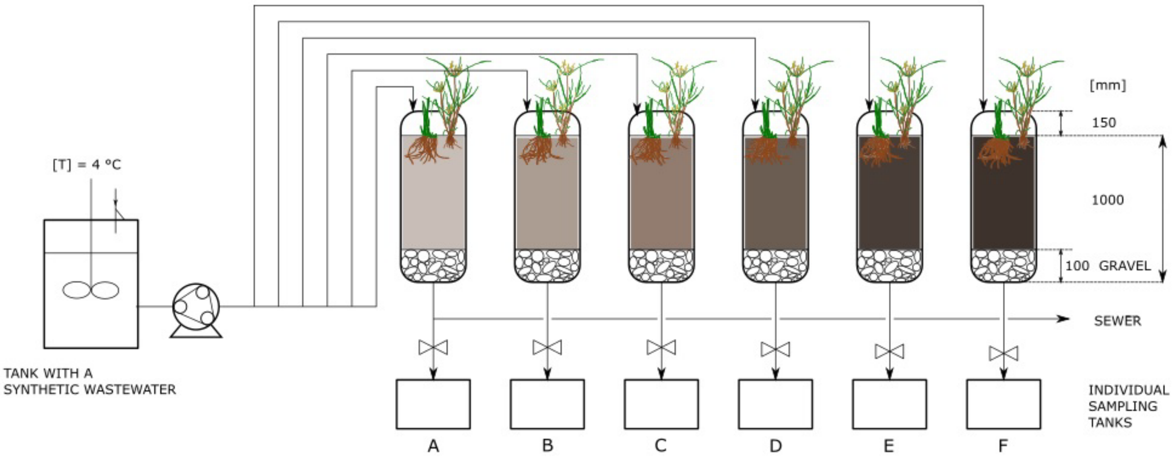


Figure 5.1 : Schematic of the laboratory installation

(sand 100 % (Column A), biochar 30 % and sand (Column B), activated biochar 15 % and sand (Column C), activated biochar 30 % and sand (Column D), zeolite 15 % and sand (Column E) and zeolite 30 % and sand (Column F))

Each column is planted with macrophytes well applied in common wetland configurations [111,232], *Phragmites australis* and *Iris pseudacorus*, combining the benefit to have long and short roots respectively.

By providing a more developed aerial and underground roots suitable for biofilm, *Phragmites australis* seemed to be especially favourable in the removal of pharmaceuticals [233,234]. *Iris pseudacorus* was selected mostly because of its strong environmental adaptability and stress resistance, but also for the reported high pollutant removal efficiencies (i.e. metals) [232].

Biochar (produced from plants (dry matter 70,23%, element contents: N 1,28%, C 80%, S 0,04%) by Palaterra, Germany) and zeolite (high sorption capacity from Zeobon, Germany) are mixed with a sand (Liapor, Germany) that serves as support (Table S0, Supplementary Information) according to the following composition: sand 100 % (Column A), biochar 30 % and sand (Column B), activated biochar 15 % and sand (Column C), activated biochar 30 % and sand (Column D), zeolite 15 % and sand (Column E) and zeolite 30 % and sand (Column F). The biochar can be activated by fermentation inoculating microorganisms such as *Lactobacillus*, *Rhodopseudomonas* and *Saccharomyces*. Column A is used as reference case, while biochar is compared with zeolite and activated biochar at the same ratios (Columns B, F and D, respectively). If the activation of biochar would show better performances, a reduction in the percentage of the cost-intensive substrate can be evaluated (Column C) in comparison to zeolite (Column E) for a better financial assessment.

The columns are operated in down flow mode and fed simultaneously and intermittently at the same flow rate, with the same synthetic wastewater influent. This results in a similar Empty Bed Contact Time (EBCT), Hourly Space Velocity (HSV) and Hydraulic Loading Rate (HLR) for each column. To ensure comparable results, a high precision six headed pump is used (Watson Marlow, Belgium). The influent synthetic wastewater is pumped at an HLR of 100 L d^{-1} m^{-2} (resulting in 0.00116 L s^{-1} m^{-2}) for 30 minutes, 3 times per day. This results in EBCT of 9 days, HSV of 216 hours and HLR of 0.004 nr h^{-1} (with nr defined as m^3 treated wastewater m^{-3} bed volume). Converted to flow rate per column, this gives 7.07 L d^{-1}. The pump is remotely regulated by a digital signal to feed columns as required. Dissolved oxygen concentrations were measured in situ using non-invasive oxygen sensors (PreSens, Germany) mounted at discrete depths across the lab-scale wetlands.

To mimic natural sunlight and to enable photodegradation, UV lamps (Megaman LED) are installed to provide 8 hours per day of light (10 a.m. to 6 p.m.).

The standard synthetic wastewater recipe Standard OECD 303 A has been adapted to meet the final values (effluent concentrations) of COD, TN, PO_4-T and NO_3-N typical of a small/medium-sized WWTP. Tap water is mixed with 45 mg L^{-1} peptone, 35 mg L^{-1} meat extract and 8 mg L^{-1} urea, 3 mg L^{-1} of KH_2PO4 as P source. This results in COD of 60 mg L^{-1}, TN of 15 mg L^{-1}, PO_4-P of 2.5 mg L^{-1}, NO_3-N of 5 mg L^{-1}. The influent water is stored in a 225 L reservoir tank (Lely Center, Greece), and continuously mixed to ensure the solution is homogenous. The tank is kept at a constant temperature of 4° C to minimize the formation of biofilm.

The experiment is conducted during 475 days and the synthetic wastewater spiked with the target contaminants (high purity standards TechLab, France) (1 to 5 ug L^{-1},Table 2.1) after the system has reached steady conditions (last 357 days). In none of the lysimeters the biofilm has been inhibited. A sampling and monitoring protocol was established. Influent grab samples were taken weekly from the storage tank to verify the stability of synthetic influent as the spiked analyte concentrations. Effluent samples from the lysimeters were taken as 24 hrs composite sample once a week.

For most spiked compounds, concentrations are in the same order of those found in the effluents of the WWTPs discharging in the Sûre river (i.e. 3 ug L^{-1} benzotriazole and diclofenac, 2 ug L^{-1} tolyltriazole in a 36000 PE WWTP effluent) [206]. Chemo-physical properties of target compounds are given in Table S1 (Supplementary Information).

5.2.2.2 Pilot-scale wetlands set up (Reisdorf)

The biological sewage treatment plant of Reisdorf-Wallendorf was commissioned from the Syndicat Intercommunal de Depollution des Eaux Residuaires du Nord (SIDEN) and the Südeifelwerke Irrel AöR in Reisdorf. It is the first Biological Combined System (BIOCOS) within the project „Internationale Abwassergruppe Untere Our“ and it came operational in June 2012. It has a capacity of 4600 PE and discharges in the Sûre river fulfilling the qualification of an innovative project (high stability, space-saving and energy efficiency) and complying with the national law restrictions for the discharge. For this, it has been considered an excellent candidate to further test the three most promising substrates of the lab scale lysimeters under real conditions.

Three cubic tanks resulting in 1 m^2 surface area each were established and operated for 6 months. The vertical flow wetlands contained from bottom to top two layers of gravel as drainage system, a substrate (sand 100 % in VFCW(A), activated biochar 15 % and sand in VFCW(C), zeolite 15 % and sand in VFCW(E)) and finally a peat layer for insulation (Figure 5.2.).

The units were planted immediately after their establishment at a density of 25 plants stems per m^2 with common reeds *Phragmites australis* and *Iris pseudacorus*, distributed alternatively. The effluent of the WWTP was used as influent to the units, pumped and distributed through a perforated plastic pipe. This pipe was placed above the wetland, lying on the peat layer in a serpentine configuration in order to ensure the most uniform water distribution. At each loading (in downflow intermittent regime), the wastewater flooded the wetland surface, drained by gravity through the wetland body and collected in a 35 L plastic tank placed at bottom of each unit.

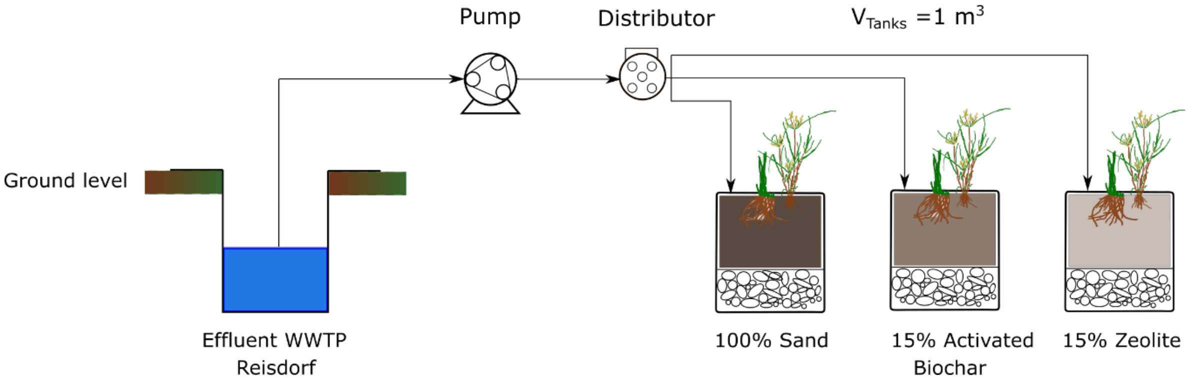


Figure 5.2 : Schematic of the installation in Reisdorf

The feeding strategy consisted on three equal daily water cycle (every 8 hrs for 30 minutes) for an applied HLR of 180 L d^{-1} m^{-2} (months 1 to 2), 230 L d^{-1} m^{-2} (month 3 to 4) and finally 300 L d^{-1} m^{-2} (month 5 to 6). The Organic Loading Rate (OLR) ranged from 1.3 to 119 g COD d^{-1} m^{-2} during the 6 months of operation.

Wastewater samples were monthly collected as influent of the WWTP (24 hrs composite sample), effluent of the WWTP (24 hrs composite sample) which is the influent to the three vertical flow wetlands and effluent from the three vertical flow wetlands (grab samples) in order to assess the performance of the WWTP and the impact of the VFCW respectively.

5.2.3 Analytical methods

5.2.3.1 Macropollutants

Common parameters were routinely monitored. COD, TN, PO_4-P, NH_4-N and NO_3-N were measured with Hach Lange cuvette tests. Oxidation-reduction potential, pH and conductivity were collected with conventional WTW (Xylem, UK) probes.

5.2.3.2 Micropollutants

The analyses of the pharmaceuticals were performed externally (Luxembourg Institute of Science and Technology LIST, Luxembourg) in two steps: enrichment by Solid Phase Extraction (SPE) and analysis of the SPE extracts by LC–MS/MS. The analytical method found in the literature [235] was adapted to the specific compounds (Table S2, Supplementary Information): acid (pH 3) for X-ray media with resin-based sorbent ENV+ cartridges and OASIS reversed-phase sorbent Hydrophilic Lipophilic Balanced (HLB) cartridges for all the other compounds. The methanol extracts were then rebuilt according to specific recoveries compound related into acetonitrile (i.e. ciprofloxacin, clarithromycin, erythromycin, lidocaine, sulfhametoxazole) or methanol (i.e. all the others) before being analysed by LC-MS/MS, an Agilent 1200 SL coupled with a Sciex Qtrap 4500 triple quadrupole with electrospray ionization (ESI) in positive mode. The analysis of glyphosate and AMPA were performed using chemical derivatization and on-line SPE-LC-MS/MS.

5.3 Results and discussion

5.3.1 Behaviour of MPs under controlled conditions (Lab-scale investigation)

The six lysimeters planted with common reeds were operated under the same conditions but with different substrates for over 357 days.

Macropollutants' data (Table S3 in Supplementary Information) indicates that the system is able to provide a consistently high COD removal efficiency (above 90 %) with concentrations below 5 mg L^{-1} and excellent stable effluent quality with both pH and conductivity uniform.

The lysimeters had a poor but constant TN (around 12 %) removal, which was expected as the CWs were operated mainly under aerobic conditions, so no denitrification took place. The excellent reduction of ammonia NH_4-N (effluent concentrations below an average value of 0.05 mg L^{-1}) together with the nitrate NO_3-N increase (effluent concentrations with average value of 7 mg L^{-1}), implicates well working nitrification process of autotrophic bacteria in all units. These findings are confirmed from consumed dissolved oxygen along the depth of the lysimeters monitored with in situ not invasive sensors which can also be related to enhanced oxygen transfer to the plant roots. Macro redox zones were not identified and oxidation-reduction potential was found continuously high confirming that an aerobic environment prevails in vertical flow wetland configurations [236].

Phosphorus removal efficiency was relevant with the highest reduction observed in Column A. This can be explained by the nature of the sand, known to be rich in Fe. Phosphorus may be retained from the substrate due to precipitation with Fe rather than adsorbed. Lysimeters with higher volume of sand are thus performing better (i.e. A, C and D) in terms of phosphorus removal. Removal values are lower in non-activated biochar (Column B) where phosphorus was initially released (Figure S.3 in Supplementary Information) and a negative removal rate observed. A standard isotherm test was carried out to confirm the result. As consequence, it was concluded that increased concentration of phosphorus in effluent was deriving from the substrate composition itself. The biochar used in this study was in fact produced through a medium temperature pyrolysis of plants (600 – 800 °C). After that, biochar results as a carrier of the phosphorus of the plants which can be released in the lysimeter effluent by leaching. When activated by fermentation, the phosphorus present in the biochar is used from the activating bacteria and thus not released into the effluent.

The results also show increased effluent conductivity values for all lysimeters mainly due to the substrate-biofilm interactions, which results in soluble salt release.

Micropollutants' eliminations are evaluated especially with respect to the type of substrate. The individual contribution of the different removal mechanisms (sorption, bio-, phyto- and photodegradation) is not further investigated as this investigation only examines the concentration of contaminants in water phase and neglects the characterisation of biofilm or plant tissues. The global eliminations are discussed and an attempt to determine possible driving elimination process is made.

Overall, the results showed high removal efficiencies (above 90 %) across the six lysimeters. The performance of the treatment step can be clustered based on compound behaviour as following (Table 5.2, S.4 in Supplementary Information):

- *Compounds with high and stable elimination for 16 out of the 27 spiked MPs*

Among them, compounds known to be persistent or difficult to be removed in conventional activated sludge systems because poorly biodegradable (i.e diclofenac, carbamazepine, diuron with less than 20

% removal) [2] showed a stable elimination profile over one-year observation time. A recent study indicated carbamazepine as removed primarily by adsorption to the surface of activated carbon and biochar. A negligible elimination of this compound was previously observed in vertical flow reeds operated with conventional gravel or sand biofilter [237]. As differences among the substrates are not detectable, the elimination of those compounds seems to finally benefit from a biological removal mode driven by organisms (bacteria, fungi supported by enzymes, bio-tissues) and including biotransformation, biosorption, bioaccumulation, bioprecipitation and biosolubilisation. The operation of studied lysimeters resulted in enhanced elimination through adsorption due to the prevalent aerobic conditions. The presence of active biofilm may potentially extend the lifetime of the adsorbent material and allows longer and stable elimination.

- *Compounds with high but unstable elimination for 6 out of the 27 spiked MPs*

Compounds show still high average rates but fluctuating over the observation time. In the case of TCPP (Figure 5.3) the elimination was especially efficient in the activated biochar and zeolite-based lysimeters.

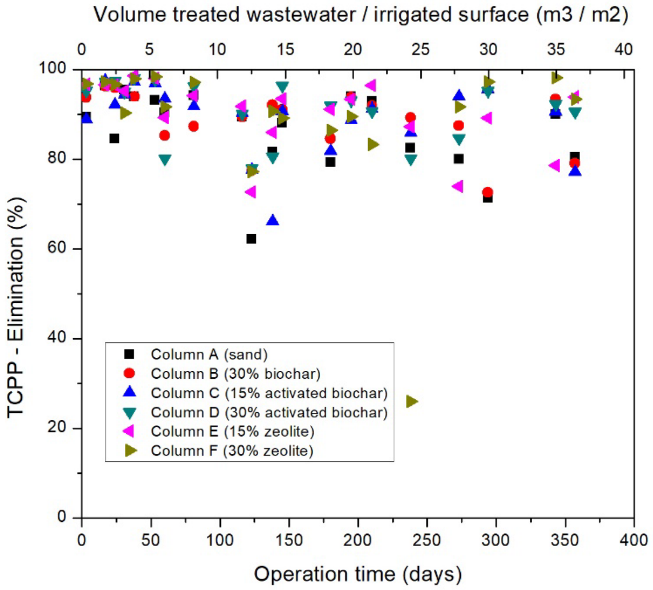


Figure 5.3 : Elimination of TCPP in the lysimeters treating synthetic wastewater during 357 days

In absence of an activated biofilm (Column B), the adsorption of TCPP declined probably due to the completion for adsorption surface between organic matter and the other micropollutants. TCPP resulted to be eliminated in smaller extend with the sand substrate (Column A) and those lysimeters where the proportion of sand towards adsorbing material was higher (Columns C and E).

Table 5.2 : Elimination rates in the lysimeters

Compound		Column A			Column B			Column C			Column D			Column E			Column F		
		Min	**Max**	**Average**	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**
high and stable elimination	Atenolol	94.6	99.7	99	89.1	99.7	98.7	89.2	99.7	98.6	95.8	99.7	99	98.6	99.7	99.3	94.9	99.7	98.9
	Bezafibrate	96.4	100	99.6	99	100	99.8	96.3	100	99.6	97	100	99.7	99.5	100	99.9	92.6	100	99.4
	Carbamazepine	96.9	99.9	99.6	93.8	99.9	99.5	99.5	99.9	99.8	95.3	99.9	99.6	99.2	99.9	99.8	91.5	99.9	99.3
	Clarithromycin	91.5	99.9	98.5	81.7	99.9	98.4	90.3	99.9	98.7	95.5	99.9	99.1	97.9	99.9	99.4	89.2	99.9	98.7
	Diclofenac	96.1	100	99.5	98.8	100	99.8	98.8	100	99.8	98.2	100	99.8	98.8	100	99.8	92	100	99.3
	Ketoprofen	98	100	99.7	98.7	100	99.8	98.7	100	99.9	97.5	100	99.7	96.2	100	99.7	91.9	100	99.3
	Lidocaine	96.8	100	99.7	98.7	100	99.9	98.8	100	99.9	98.6	100	99.8	98.7	100	99.9	91.6	100	99.2
	Metoprolol	92.1	100	99.2	98.7	100	99.8	99.2	100	99.9	96.2	100	99.7	99.2	100	99.9	82.6	100	98.9
	Propranolol	95.6	99.9	99.5	98.4	100	99.8	98.4	99.9	99.8	98.2	100	99.8	98.4	100	99.8	92.7	100	99.5
	Benzotriazole	92.4	99.9	98.7	96.5	99.9	99.2	96.8	99.9	99.2	93.8	99.9	99.1	86.4	99.9	98.6	-60.6(90)	99.9	90(98.3)
	Carbendazim	96.8	99.9	99.5	98.5	99.9	99.6	98.9	99.8	99.6	99.5	99.9	99.7	86.5	99.8	98.2	90.7	99.9	99.1
	Diuron	94.9	99.8	99.2	95	99.8	99.4	95.2	99.8	99.4	94.6	99.8	99.4	95	99.8	99.4	90.5	99.8	98.9
	Isoproturon	92.1	99.8	99.2	92	99.8	99.3	92.3	99.8	99.3	91.2	99.8	99.2	92	99.8	99.3	90.7	99.8	98.8
	Terbutryn	96.6	99.9	99.5	96.5	99.9	99.7	96.6	99.9	99.7	96.2	99.9	99.6	96.5	99.9	99.6	-71(95.7)	99.9	91(99.4)
	Tolyltriazole	96.5	99.9	99.5	96.5	99.9	99.6	96.6	99.9	99.6	96.1	99.9	99.6	96.5	99.9	99.6	90	99.9	99
	Glyphosate	98.2	99.9	99.7	99.4	99.9	99.8	99.4	99.9	99.9	98.3	99.9	99.8	94.1	99.9	99.6	95.3	99.9	99.4
high but unstable elimination	Ciprofloxacin	75.3	99.7	95.3	77.9	99.7	96.1	74.9	99.7	96.1	76.2	99.7	96.2	75.7	99.7	96.2	75.7	99.7	94.2
	Erythromycin A	93.5	99.8	98.2	95.5	99.8	98.3	95.7	99.8	98.4	95.8	99.8	98.4	95.5	99.8	98.4	92.3	99.8	98.2
	N-acetyl sulfamethoxazole	7.3	88	76.4	5.6	88.5	76.2	4	87.3	75.8	7.3	88.7	76.2	15.3	88.4	76.2	12.1	89.3	76.7
	Sulfamethoxazole	97.8	99.9	99.6	96.9	99.9	99.6	97.8	99.9	99.7	97.4	99.9	99.6	97.7	99.9	99.7	93.6	99.9	99.2
	DEET	96.9	99.9	99.4	93.8	99.9	98.6	95.8	99.9	99.3	98	99.9	99.4	97.7	99.9	99.4	50.8	99.9	95.8
	TCPP	62.1	96.4	86.6	-36(28.5)	96.2	79.9(85)	66.1	97.4	89.2	77.9	98	90.4	72.7	98.6	89.8	26	98.4	88.2
medium elimination	Cyclophosphamide	3.2	99.9	77.1	9.9	98.1	57.6	30.4	99.6	74.3	41.2	99.6	80.3	18	100	76.9	-3(6.7)	100	62(65.9)
	MCPP	88.8	99.8	98.6	61.5	97.6	87.7	94.3	99.7	98.6	91.7	99.7	99	89.7	99.8	98.8	72.5	99.8	94.8
	AMPA	21	99.3	78.6	9.4	94.8	55.6	17.1	98.5	73.7	29.8	98.4	70	39.6	99.3	83.7	-55	99.3	69 (75.6)
	PFOS	17.2	99.9	89.5	-15.1	99.7	66.1	29	99	85.9	46.7	99.9	87	-20	99.9	89.3	-94.8	99.8	82.2
	PFOA	-33.4	96.6	51.4	-43.4	93.3	35.6	-28.7	93.6	42.4	-25.6	89.6	45.7	-33.1	95.8	51.3	-66.5	99.9	54.8
	Average/Substrate			**94**			**91**			**94**			**94**			**95**			**93**

This may be related to the smaller specific surface area of sand in comparison with to the sand mixed with biochar or zeolite.

- *Compounds with medium elimination for the remaining 5 spiked MPs*

Differences can be detected among the substrates with a general tendency towards better performances for activated biochar and zeolite-based lysimeters. While glyphosate is highly removed from all substrates during the observation time in line with literature as it is known to be adsorbed by clays and organic matter [238], the adsorption of its metabolite AMPA declined towards lower elimination rates (

Figure 5.4 and Figure 5.5).

Glyphosate is expected to be rapidly released from these adsorption sites by the competence with inorganic phosphates [238,239]. The microflora present in the substrate would then degrade glyphosate to AMPA as metabolite which would ideally accumulate in the substrate giving preference to sorption mechanism [238,239].

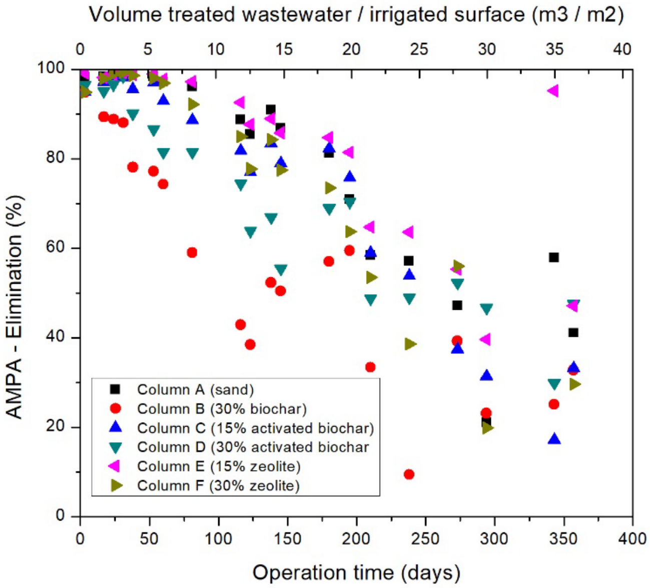


Figure 5.4 : Elimination of AMPA in the lysimeters treating synthetic wastewater during 357 days

AMPA in fact contains phosphate known to be adsorbed onto mineral surface [240]. The elimination of AMPA is progressively decreasing showing a path independent from the constant load of the feeding and suggesting a limited capacity for the selected substrates towards this compound. Generally, zeolite-based substrates showed a better affinity which can be boosted from the higher cation exchange capacity of this material.

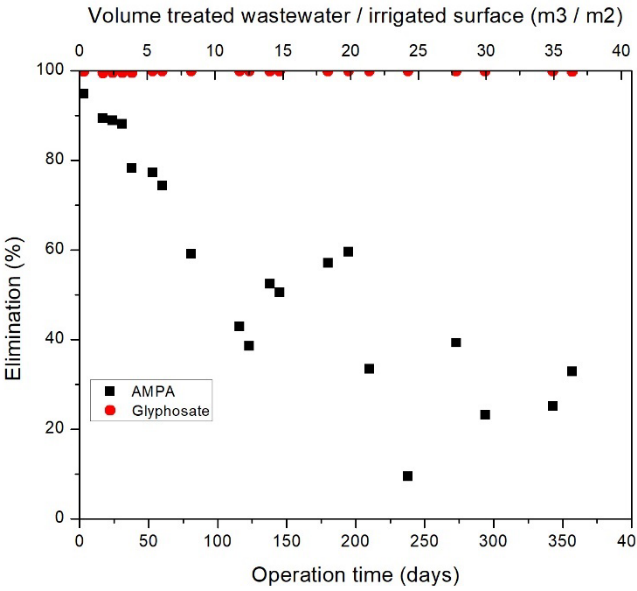


Figure 5.5 : Elimination of AMPA towards glyphosate in Column B treating synthetic wastewater during 357 days

Some understanding about the possible sorption process can be derived by exploring how experimental data may fit one of the most popular models, the Freundlich one. The Freundlich equation has an empirical origin, but can be derived from the Langmuir isotherms and thus it is able to describe the complexity of the sorption process [241]. In this case, the excellent fit to the Freundlich equation reflects the heterogeneity of the sorbent material as well as possible interactions between the adsorbed molecules.

The adsorption of AMPA to the surface of these materials can be described equation (5.1), which defines the relation between the loading q of the substrate (amount of adsorbate adsorbed per g of adsorbent) [242] and the operation time at which the effluent concentration is measured. K and n can denote the adsorption parameters.

$$q = \mathrm{K}\, T^{1/n} \quad (2.1)$$

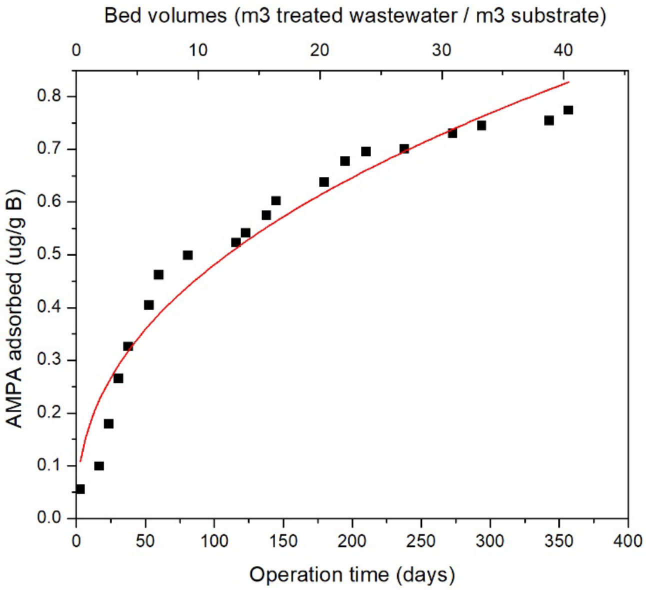


Figure 5.6: Breakthrough curve of AMPA in biochar based lysimeter treating synthetic wastewater during 357 days. Spiked concentration: 5000 ng/L

Breakthrough is shown as function of the lysimeters operation time and the adsorbed concentrations are normalized by the weight of the individual substrate (cumulative curve, Figure 5.6). After 250 days of operation AMPA broke through to 60 % defined as c/c0 [243] where c is the effluent and c0 the influent concentration, and equivalent to 40 % elimination.

Cyclophosphamide and MCPP depicted similar behaviour (S5 in Supplementary Information). Adsorption parameters for the three compounds mentioned above are collected in Table 5.3.

Table 5.3: Adsorption parameters of mentioned compounds after adsorption from synthetic wastewater on sand 100 % (Column A), biochar 30 % and sand (Column B), activated biochar 15 % and sand (Column C), activated biochar 30 % and sand (Column D), zeolite 15 % and sand (Column E) and zeolite 30 % and sand (Column F)

	AMPA				**Cyclophosphamide**				**MCPP**			
	K	n	1/n	R_2	K	n	1/n	R_2	K	n	1/n	R_2
Column A	0.0630	2.0303	0.4925	0.9599	0.0630	2.0303	0.4925	0.9599	0.0039	1.4682	0.6811	0.9908
Column B	0.0677	2.3482	0.4259	0.9456	0.0626	2.2585	0.4428	0.9741	0.0042	1.5286	0.6542	0.9879
Column C	0.0635	2.0656	0.4841	0.9566	0.0598	2.0413	0.4899	0.9791	0.0039	1.4686	0.6809	0.9909
Column D	0.0626	2.1112	0.4737	0.9629	0.0521	1.9141	0.5224	0.9868	0.0038	1.4613	0.6843	0.9911
Column E	0.0581	1.9515	0.5124	0.9692	0.0605	2.0259	0.4936	0.9786	0.0039	1.4647	0.6827	0.9909
Column F	0.0695	2.1449	0.4662	0.9437	0.0686	2.2824	0.4381	0.9739	0.0036	1.5481	0.6460	0.9597

The reproducibility of the data used to generate the adsorption coefficients can be judged by means of the regression coefficient (square of the Pearson Product-Moment Correlation Coefficient) R_2. The closer to 1 the value is then the more reproducible the data used to calculate.

The value of the adsorption constant (K, denoted as capacity factor) can be useful in gauging the difference in the adsorption capacity of a given material for a range of compounds or the adsorption capacity of a given compound for a range of materials, as long as the exponent value (1/n, denoted as intensity parameter) is relatively constant [244]. The heterogeneity of K and n however does not allow an immediate comparison among the substrates.

Considering that the individual compound kinetic follows a pseudo-first order approximation along the time of operation, the exponential distribution of each compounds with respect to the individual substrates has been determined by curve fitting using the data in Table 2.3 so that the results of the substrates can be easily compared (Figure 2.7 and S6 in Supplementary Information).

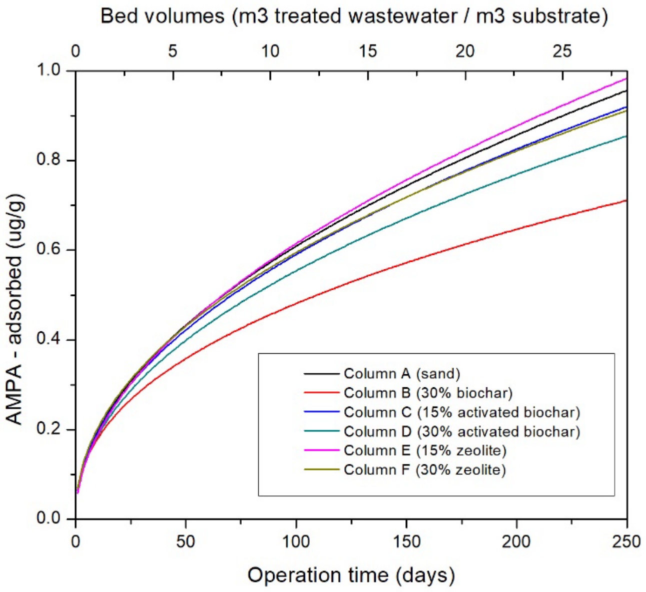


Figure 5.7 : Modelled AMPA degradation kinetics in lysimeters treating synthetic wastewater during 250 days. Spiked concentration: 5000 ng/L

According to the only coefficient displayed in Table 5.3, AMPA seems to be well adsorbed in lysimeters A and C with higher K at given n. However, from the modelled degradation kinetic, column E depicted a slightly better performance by having lower calculated K and n coefficients. Once the plateau has been reached after 250 days of operation, Column B has treated 2.7 m^3 of synthetic wastewater adsorbing around 0.77 ug of AMPA per g of substrate in comparison to 1 ug of AMPA adsorbed per g of A and C. Similarly, cyclophosphamide showed a slight preference for Column C, 1 ug of active ingredient has been adsorbed per g of substrate C in comparison to 0.7 ug adsorbed per g of B. MCPP showed the lowest affinity for adsorption reaching 0.2 ug of active ingredient adsorbed per g of substrates with the only exception of B and F.

PFOS (Figure 5.8) showed high and stable eliminations during the first 100 days of operation and a general decay thereafter, with lower elimination in the sand (Column A) and non-activated biochar (Column B). This may indicate that the removal mechanism for this compound is a combination of adsorption and biodegradation as high elimination are observed when a higher percentage in volume of activated biochar is applied (from Column C to D). PFOA (Figure 5.9) confirmed a similar behaviour depicting a low elimination for sand and non-activated biochar towards better removal when higher percentage in volume of activated biochar is applied. Still biological degradation may be less effective for PFOA in comparison to PFOS.

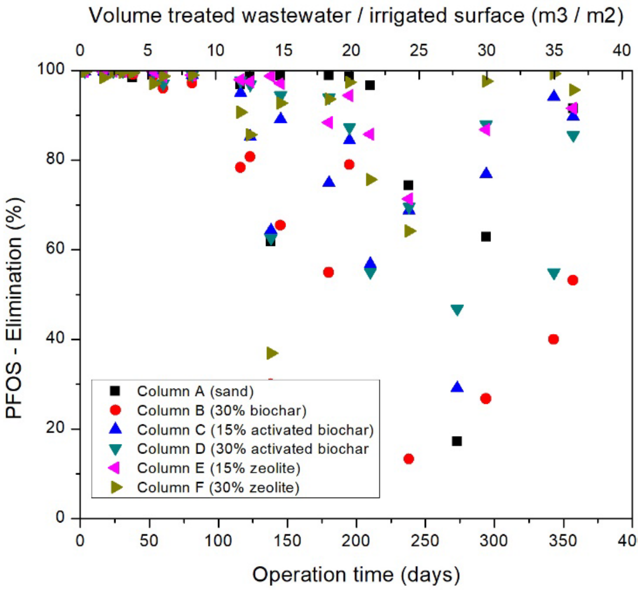


Figure 5.8 : Elimination of PFOS in the lysimeters treating synthetic wastewater during 357 days

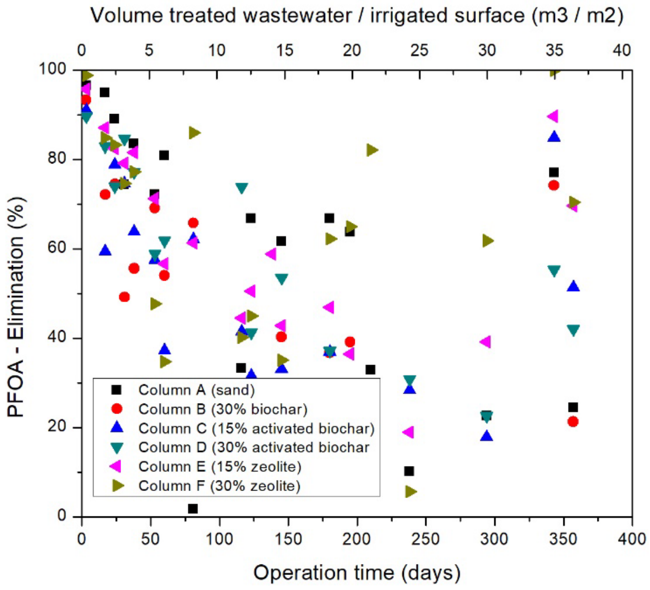


Figure 5.9 : Elimination of PFOA in the lysimeters treating synthetic wastewater during 357 days

For both compounds the effluent of the lysimeters contained in few occasions (after 138 and 273 days of operation) higher concentration of active ingredient in comparison to the influent (Figure 5.10).

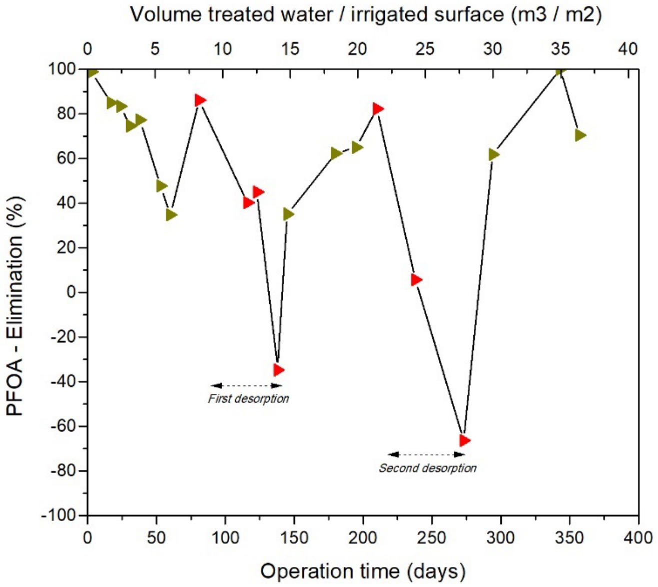


Figure 5.10: Elimination of PFOA in Column F (30% zeolite) treating synthetic wastewater during 357 days: two desorption events are identified and displayed with red data points (138 days, 273 days)

Such negative removal can be explained by desorption of the accumulated compounds on the substrates which can be only temporary retained. The rapid increase in elimination thereafter suggests a 'regeneration' of the sorption site newly available. Similar behaviour has been observed in a previous study [245].

5.3.2 Behaviour of MPs under variable conditions (Pilot-scale investigation)

5.3.2.1 Occurrence and removal efficiencies of 27 selected MPs in the WWTP of Reisdorf

The occurrence of the 27 selected compounds was investigated at the influent and effluent of WWTP Reisdorf in order to validate laboratory results and evaluate the removal efficiencies and the impact of a possible polishing step with VFCWs. Average concentrations of each compound together with WWTP removal efficiencies are reported in Table 5.4. The standard deviation quantifies the high variability between sampling days with respect to the number of analysed samples (N).

Table 5.4: Average concentrations (± Std) measured during the observation period (6 months) for 27 compounds (ng L^{-1}) and WWTP removal efficiencies (%), N=11

Compound	**WWTP**		
	Influent (ngL^{-1})	**Effluent (ngL^{-1})**	**Removal (%)**
Atenolol	912±258	50±23	95
Bezafibrate	736±137	16±11	98
Carbamazepine	120±119	139±12	0
Clarithromycin	360±172	168±25	54
Ciprofloxacin	537±307	152±89	72
Cyclophosphamide	<LOQ	<LOQ	n.c.
Diclofenac	2374±946	995±446	58
Erythromycin	<LOQ	<LOQ	n.c.
Ketoprofen	215±149	10±10	95
Lidocaine	59±22	72±50	0
Metoprolol	323±126	367±149	n.c.
Propranolol	41±35	78±37	n.c.
N-acetyl sulfamethoxazole	139±306	23±27	83
Sulfamethoxazole	133±277	59±57	56
Benzotriazole	5308±1336	1571±701	70
Carbendazim	<LOQ	<LOQ	n.c.
DEET	1279±1090	108±11	92
Diuron	<LOQ	<LOQ	n.c.
Isoproturon	<LOQ	<LOQ	n.c.
Terbutryn	<LOQ	<LOQ	n.c.
MCPP	<LOQ	<LOQ	n.c.
TCPP	9672±2589	3076±1080	68
Tolyltriazole	1508±424	761±212	50
Glyphosate	1297±2508	477±840	63
AMPA	1175±263	2195±1001	-87
PFOS	96±25	15±16	84
PFOA	39±15	12±9	69

Among 27 MPs, 7 compounds were not detected in relevant concentrations in both influent and effluent of the WWTP due to the following possible reasons:

- cyclophosphamide: among cytostatics, cyclophosphamide is not the most administrated one and presents a number of metabolites and transformation products not considered in this study. Only 10% of the administrated active ingredient is excreted unchanged via urine;

- erythromycin: among macrolides this antibiotic mostly suffers from analytical detection problem. Also, only 5% of the administrated active ingredient is excreted unchanged via urine;
- carbendazim: the EC does not approve carbendazim as an active substance for use of biocidal products of product-type 9 reducing strongly its application;
- diuron: the use of this substance has been limited by the EC and is under assessment as endocrine disruptive;
- isoproturon: the application was not renewed from the EC in 2016 due to its eco-toxicity relevance and carcinogenic potential;
- terbutryn: the low concentration indicates that wastewater treatment plants are not relevant sources of emission; however higher concentrations can be expected in summer during the main season of pesticide use. A possible high level in surface bodies must be thus related to other factors (i.e. Combined Sewer Overflow (CSO), diffuse emission from agriculture etc);
- MCPP: concentration of mecroprop are varying starting with the use (manufacturer garden and household) and with climate changes (increased temperatures, seasonal variability, humidity and rainfalls).

As consequence, it was not possible to calculate the WWTP removal efficiency (n.c. not calculated) for the compounds mentioned above (Table 5.4).

For few compounds, the effluent concentrations were higher than the influent ones. The reasons for that could be linked to the experimental methodology (i.e. analytical and sampling) or to the fate mechanism of the single compound:

- carbamazepine and lidocaine are known to be persistent and hardly removed [2]; [206] in Conventional Activated Sludge treatments. Additionally, its metabolites can build back to the parent compound [246–248]. A zero removal is thus assumed;

- metoprolol and propranolol are usually present in wastewater in highly varying concentrations because used irregularly from the patients compared to their companion atenolol, affecting severely the interpretation of the results;
- AMPA is a transformation product of glyphosate and thus its fate has to be related to its parent compound.

Benzotriazole, TCPP, glyphosate, diclofenac, tolyltriazole and DEET were detected as the most abundant in the WWTP influent exceeding 1000 ngL^{-1} average concentrations. Among them:

- DEET was highly removed (over 90 %), together with atenolol, bezafibrate and ketoprofen;
- benzotriazole, TCPP, tolyltriazole and diclofenac where moderately removed (40 % < removal < 90 %), together with the highly administrated antibiotics clarithromycin and ciprofloxacin.

High removal efficiencies are not always related to low final concentrations of the selected compounds thus the need of a polishing step has to be contextualised.

WWTP effluent concentrations of TCPP, tolyltriazole, diclofenac are relevant. Clarithromycin, discharged with an average concentration of 168 ng L^{-1} from Reisdorf WWTP, has been introduced in the Watch List (EC) 2015/495 (2015) [179] with an AA-EQS 90 ng L^{-1}.

5.3.2.2 *Removal efficiencies of 27 selected MPs in the Pilot-scale installation of Reisdorf*

Table 2.5 reports the average removal efficiencies of each investigated compound where the three substrates are applied over 180 days of operation. The influent to the three VFCWs is equal to the WWTP effluent. Among 27 micropollutants, 18 compounds were considered relevant.

Table 5.5: Average concentrations (± Std) measured during the observation period (6 months) for 18 compounds (ng L^{-1}) and VFCWs efficiencies (%), N=11

Compound		**VFCW(A)**		**VFCW(C)**		**VFCW(E)**	
	Influent (ngL^{-1})	**Removal (%)**	**Effluent (ngL^{-1})**	**Removal (%)**	**Effluent (ngL^{-1})**	**Removal (%)**	**Effluent (ngL^{-1})**
Atenolol	50±23	49	25	54	23	53	24
Carbamazepine	139±12	79	29	92	11	84	22
Clarithromycin	168±25	93	12	96	7	96	7
Ciprofloxacin	152±89	78	33	82	28	81	29
Diclofenac	995±446	97	25	99	12	98	21
Lidocaine	72±50	94	4	96	3	96	3
Metoprolol	367±149	92	31	97	10	96	16
Propranolol	78±35	91	7	94	4	94	4
N-acetyl sulfamethoxazole	23±27	77	5	83	4	79	5
Sulfamethoxazole	59±57	24	45	73	16	11	53
Benzotriazole	1571±701	93	111	98	28	95	76
DEET	108±11	64	39	78	24	46	58
Tolyltriazole	761±212	97	24	99	9	98	18
Glyphosate	477±840	96	17	93	35	94	27
AMPA	2195±1001	81	414	59	894	71	639
TCPP	3076±1080	29	2172	28	2219	30	2149
PFOS	15±16	48	8	45	8	44	8
PFOA	12±9	20	10	18	10	29	9
Total average Removal (%)		**71**		**76**		**71**	
Total average Removal excluding Fluoro-surfactant (%)		**75**		**81**		**74**	

When the average removal rate is compared (Figure 5.11), the substrate with activated biochar (VFCW(C)) shows the best removal rates towards the selected compounds with special attention to the highly administrated antibiotics (clarithromycin and ciprofloxacin), beta-blockers, diclofenac and tolyltriazole.

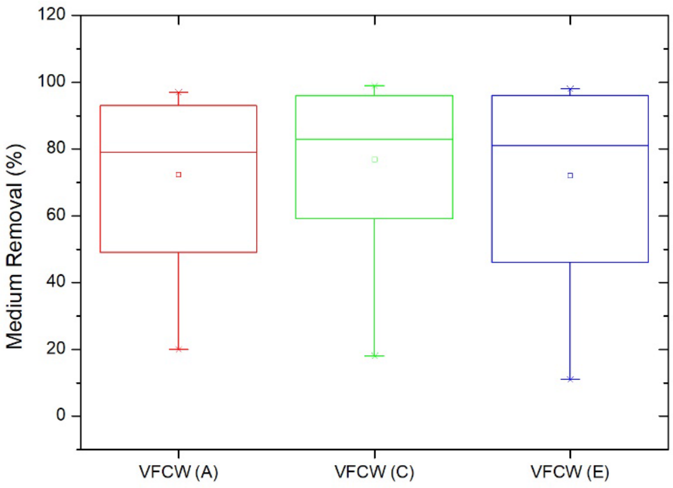


Figure 5.11 : Average removal efficiencies of the three VFCWs

Overall, none of the VFCWs was able to remove further the flame retardant TCPP. A relevant concentration in the effluent is detected (above 2000 ng L^{-1}). Same conclusion for AMPA which has to be related to its parent compound glyphosate.

When compared with the results of real matrix, laboratory experiments show to be a robust approach that can give indications for compounds not relevant in our domestic wastewater (i.e. isoproturon, diuron, etc.) but that may be relevant for CSOs and run off waters. Eliminations for activated biochar substrate in both synthetic and wastewater matrices are selected paying attention to those compounds present in relevant concentrations in the effluent of the WWTP of Reisdorf and classified as compounds with high and stable elimination for the lab-scale lysimeters. This selection allowed to compare the removal elimination in wastewater matrix with the stable average removal elimination from laboratory scale (Figure 5.12).

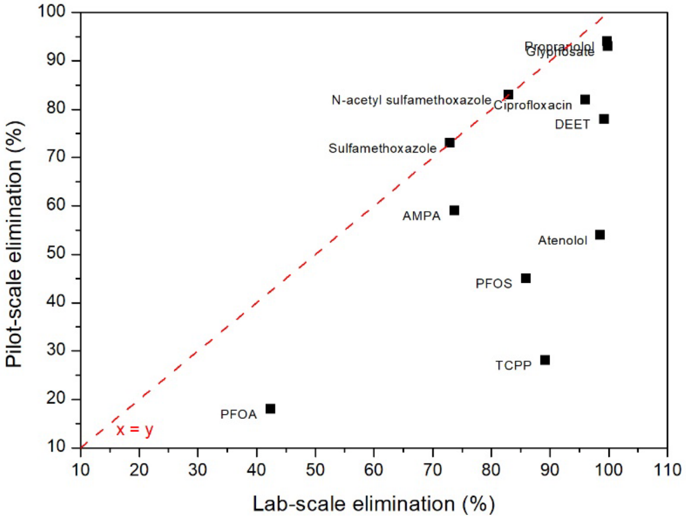


Figure 5.12 : Pilot versus Lab-scale data (above 95 % elimination not displayed)

Results show:

- excellent agreement (delta less than 6 %) between laboratory and pilot plant for benzotriazole, clarithromycin, diclofenac, glyphosate, lidocaine, metroprolol, propranolol, tolyltriazole;
- a good agreement (delta between 6 and 25 %) for AMPA, carbamazepine , ciprofloxacin and DEET;
- a medium/low agreement (delta higher than 30 %) for the others;

which can indicate potentially high removal efficiencies also for those compounds (bezafibrate, carbendazim, diuron, isoproturon, ketoprofen) not present in relevant concentrations in the effluent of the WWTP of Reisdorf but classified as compounds with high and stable elimination for the lab-scale lysimeters.

For compounds like ciprofloxacin and DEET high elimination can still be expected with a lower magnitude compared to laboratory results. For TCPP the impact of the matrix seemed to be higher and the competition between sorption and biodegradation stronger.

Among the compounds with medium elimination only AMPA seems to be present in relevant concentrations in the effluent of the WWTP of Reisdorf. To model the overall elimination of AMPA a single exponential function along the operation time is used. According to the modelled global degradation kinetics, AMPA seemed to be equally adsorbed from sand mixed to activated biochar 15 % in both synthetic and real wastewater matrices (similar slope of the curve, S7 in Supplementary Information).

5.3.2.3 Relative contributions of conventional WWTP and VFCW to the removal of relevant MPs

The removal contributions of the two treatment steps (conventional WWTP and additional treatment step VFCW) to the overall elimination of the selected compounds (Figure 5.13) is especially reflected in the final effluent concentrations discharged in the receiving river.

The VFCW (C) resulted to be:

- the dominant treatment in the elimination of those compounds that are known to be persistent and hardly removed in Conventional Activated Sludge treatments (i.e. carbamazepine and lidocaine);
- a strong contributor in the elimination of antibiotics (especially clarithromycin and ciprofloxacin), diclofenac, benzotriazole and tolyltriazole;
- a not significant contributor in the elimination of those compounds highly degraded from the WWTP (i.e. atenolol, DEET, TCPP and fluorosurfactants).

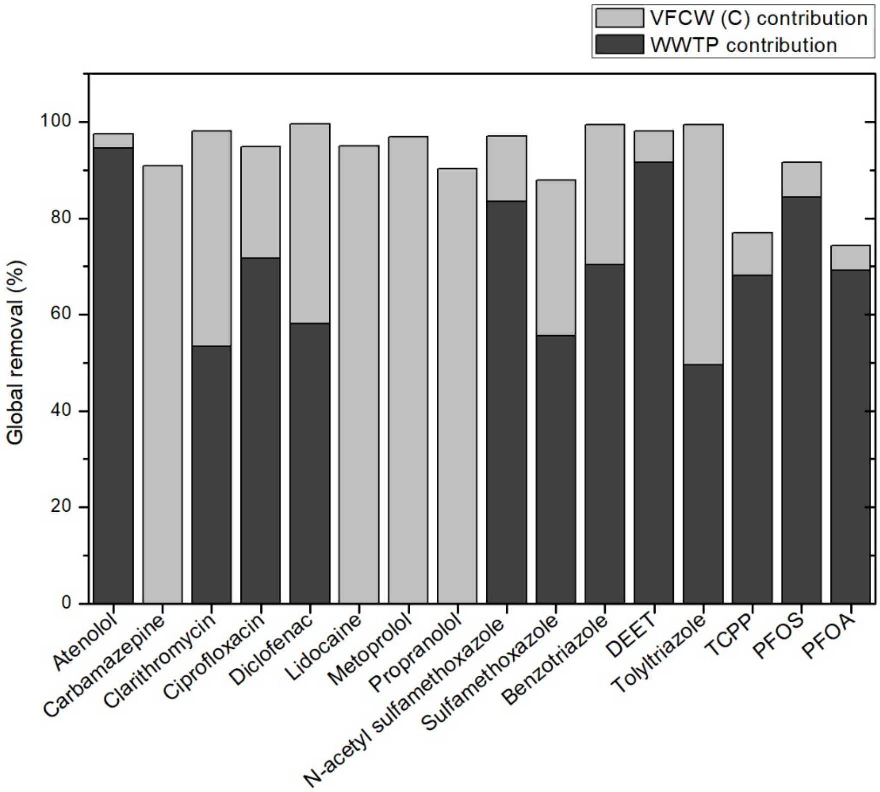


Figure 5.13: Relative contributions of the VFCW(C) and the WWTP to the overall global average removal efficiencies

In a regulatory perspective, the use of a VFCW for the WWTP of Reisdorf allows to comply with the 80 % removal threshold for the four mandatory compounds defined by Luxembourgish Water Administration diclofenac, carbamazepine, clarithromycin and benzotriazole [249] with the average removal rate of 99, 92, 96 and 98 % respectively.

5.4 Conclusions

On the basis of the data set collected from running one year six lysimeters in laboratory's controlled conditions and six months three VFCWs under real variable conditions, the following conclusions are drawn:

- generally, the majority of the selected compounds showed a high and stable removal over the observation time. In particular, compounds known to be persistent (i.e. carbamazepine and diclofenac) are proven to be well degraded in a VFCW configuration due to the chosen substrate affine to biosorption and alternative feeding regime. TCPP shows evidence of being more vulnerable as high concentrations are still detected making difficult to have conclusive statement about the most dominant mechanism for its degradation. Few compounds (i.e. AMPA and cyclophosphamide) rather showed to be poorly removed suggesting sorption as their driving mechanism. A curve fitting approach demonstrated to be a valid method to describe the process and quantify the maximum amount of active ingredient adsorbed per amount of substrate used. Column B with activated biochar has treated 2.7 m3 of synthetic wastewater adsorbing around 0.77 ug of AMPA per g of substrate in comparison to 1 ug of AMPA adsorbed per g of substrate present in columns A and C respectively.
- the occurrence and removal of selected compounds was evaluated in a VFCW configuration under real conditions. The removal of relevant emerging contaminants was higher in activated biochar substrate and confirmed good alignment with laboratory results. This comparison clearly highlights not only the robustness of laboratory scale findings but also allows to extend conclusions about compounds that are not relevant in municipal wastewater but that may be relevant for example for CSO. It is the case of biocides that are reversed in surface water via bioleaching during rain events (i.e. diuron, isoproturon etc) and that could be treated with a VFCW.
- The need end feasibility of a VFCW as polishing step to remove relevant emerging contaminants from the effluent of a conventional wastewater treatment plant has been demonstrated: more than 80 % removal has been achieved for more persistent (i.e. carbamazepine and diclofenac), highly used (i.e. benzotriazole) and eco-toxic relevant compounds.

Acknowledgements

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Supplementary information

Supplementary data to this article can be found online.

6 Publication II - Characterization of unconventional sand based substrates for adsorption of micropollutants in nature-based systems

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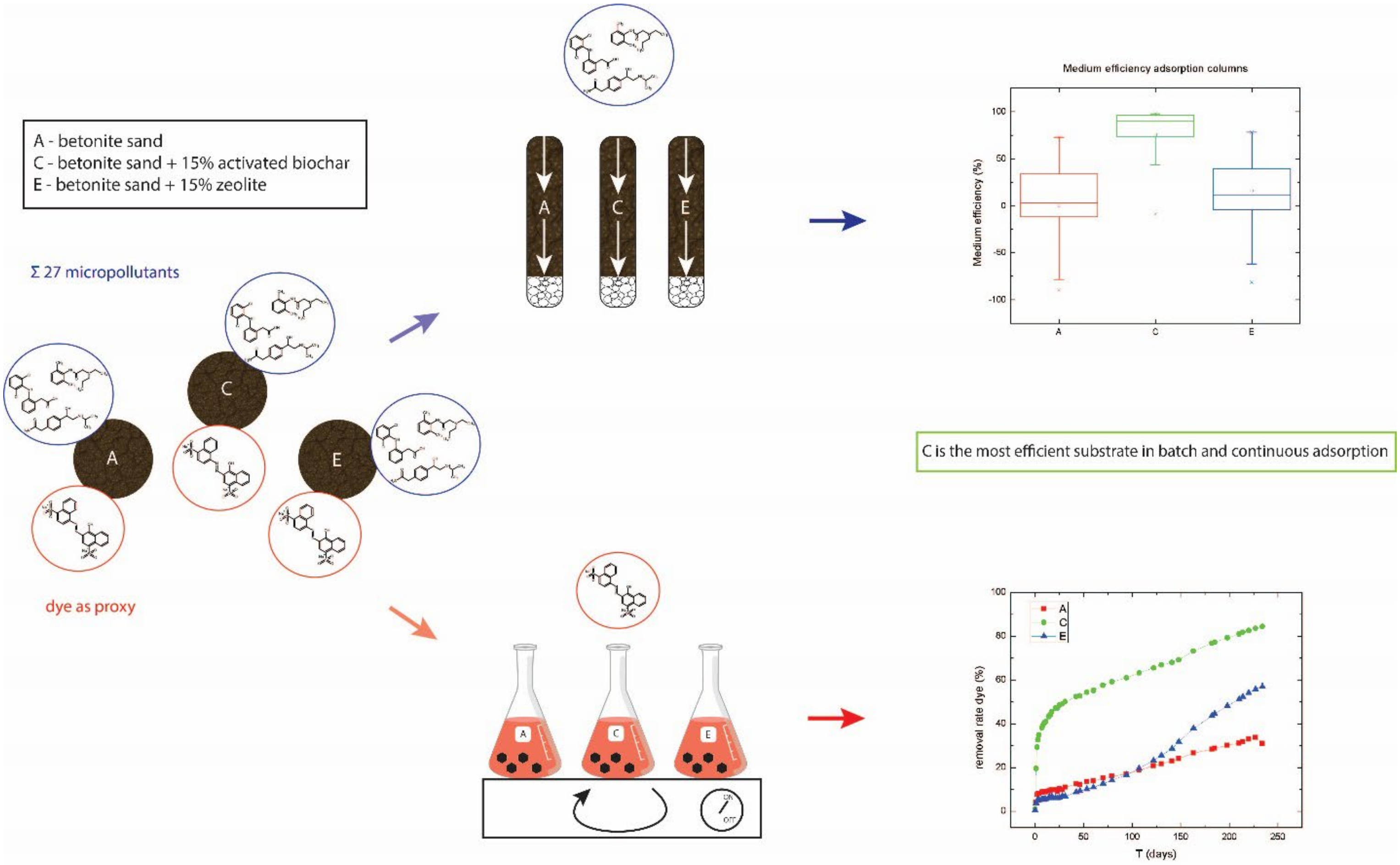
Graphical abstract



Abstract

The focus of this study is the characterization of unconventional sand-based substrates used in our previous project EmiSûre, (Interreg Greater Region (German federal states Rhineland-Palatinate and Saarland, the Grand Duchy of Luxembourg, regions Wallonia and Lorraine from Belgium and France, respectively), 2017 - 2021). The project aimed to develop and test alternative, nature-based technologies for the elimination of micropollutants (MPs) from municipal wastewater. For the characterization, two approaches were chosen. In the first approach, adsorption kinetics with a single compound allowed a perception of the adsorption capacity of the studied substrates compared to conventional substrates (granular activated carbons). This knowledge was completed by the second approach: an implementation of the studied substrates in packed-bed columns, which treated a mixture of 27 MPs in tap water for 10 months. Additionally, all three substrates (bentonite sand, sand with 15 % activated biochar and sand with 15 % zeolite) were characterized for physical and chemical properties, and the microbial potential of the activated and non-activated biochar was examined. From the studies, it is clear that the sand with an admixture of activated biochar is the most efficient sorbent in terms of single compound adsorption in batch (dye) and adsorption of 27 MPs on packed-bed columns. In contrast to the two other substrates, it shows long-term stable removal efficiencies. In the packed-bed columns, 18 out of 27 compounds were removed on average with high efficiency (80 - 99 %), which is impressive, if we consider the variety of the compounds examined (pharmaceuticals, herbicides, pesticides, etc.) and their removal in conventional treatments. Additionally, adsorption models were created for the experimental data of all compounds adsorbed on the substrate with an admixture of activated biochar resulting in the best fit with the combined Langmuir-Freundlich model. These satisfying results suggest the application of the sand-based substrate with an admixture of activated biochar for further research and possibly upscale installations with the aim to offer and prove a reasonable and efficient alternative for MPs elimination from municipal wastewater.

Keywords: unconventional substrates, adsorption processes, micropollutant removal, isotherm models.

6.1 Introduction

As a result of growing population, globalization, and anthropogenic influences, the number of pollutants in water and consequently also in wastewater has grown. It is well known that conventional municipal wastewater treatment plants (WWTPs) are not designed for the removal of so-called micropollutants (MPs) [250]. These compounds are then discharged into the aquatic environment, where they are confronted with living organisms with possible negative consequences [251], and therefore a necessity to establish an additional treatment step needs to be considered. Among commonly known technologies for advanced treatment are membrane filtration [252], advanced oxidation processes [29], or constructed wetlands (CWs). A deeper investigation of the behavior of MPs in CWs with subsurface flow configuration has previously been carried out (EmiSûre project, Interreg Greater Region (German federal states Rhineland-Palatinate and Saarland, the Grand Duchy of Luxembourg, regions Wallonia and Lorraine from Belgium and France, respectively), 2017 - 2021). Based on the promising results of this project, a further aim has been set up in the current research to determine the quantification of MPs' removal mechanisms, typically characterized by adsorption, phyto- and bioremediation [253]. The research started with a focus on phytoremediation [228] followed immediately by adsorption, which, contrary to the other mechanisms, has not yet been widely addressed concerning the application of unconventional substrates (i.e. activated biochar). Adsorption is a technology widely used for water and wastewater purification, owing to its easy applicability, efficiency, and financial availability. It is suitable for removal of a broad variety of soluble, insoluble, biodegradable, and, in general, persistent particles and it enables us to gain knowledge of different scales of the issue – from lab-scale to industrial applications in full scale.

This work aims to describe the adsorption process from two points of view; in batch experiments (kinetics with a dye) and continuous experiments (adsorption of 27 MPs on packed-bed columns). Additionally, these substrates are characterized mainly by their adsorption efficiency, followed by physical and chemical characterization and described by sorption capacities concerning common concentration ranges of MPs in real wastewater (ng/g - µg/g). The compounds studied were selected in the EmiSûre project based on various aspects: pharmaceuticals, due to their high excretion; cytostatics, due to their potential eco-toxicity; and also compounds under observation in agreement with European Commission Strategy and KomS (Kompetenzzentrum Spurenstoffe) guidelines.

For the adsorption kinetics, a single-compound approach was chosen (adsorption of one compound – dye Basovit Red 400E) to characterize the adsorption capacity of each substrate mixture (sand-based soils) as well as commercially available granulated and regenerated activated carbons (GAC & RAC). These were chosen as comparative substrates for an evaluation of the method and to compare the different efficiencies of adsorption. Activated carbons were used as reference substrates thanks to their proven excellent adsorption capacity [37,254] and broad usage in water treatment for over 80 years [255]. For substrate sterilization, it was assumed that the only removal mechanism responsible for the elimination of MPs from the liquid solution is adsorption. As the soils considered had been applied at a pilot-scale level, an entire characterization (BET (Brunauer, Emmett and Teller) specific surface area, pH, median pore diameter, porosity, mineral composition) was performed. Due to the potential use of the packed-bed columns in industry, their parameters, such as column size, dimensions, hydraulic conditions, etc., were carefully chosen and others: mass transfer zone, breakpoint, and saturation were obtained.

Sand-based substrates have already shown their successful application in subsurface treatment wetlands and similar systems [256]. For the substrates used in our investigations, applied media sand was enriched by an admixture of biochar and zeolite. Biochar used in this study was produced from a variety of lignocellulose biomasses, not just solid wood, and was pyrolyzed under 600 - 800 °C and additionally activated by anaerobic fermentation. These steps imparted the biochar with advantageous features for the adsorption process, such as high porosity, large ion exchange capacity, and great stability against biodegradation. Zeolite is a substrate commonly used for different kinds of adsorption (Wang & Peng, 2010) e.g. application in drinking water purification.

The adsorbate in the performed batch experiments was a dye Basovit Red 400E, whereas in the packed-bed columns it was a mixture of 27 previously investigated MPs, consisting of pharmaceuticals, herbicides, pesticides, corrosion inhibitors, and fluorosurfactants. However, it is important to mention that competitive group adsorption of the 27 compounds was not studied in this case, but adsorption of every single compound was separately observed, even though all the compounds were present together in one mixture. Besides the assessment of the removal rate, three well-known adsorption isotherm models (Freundlich, Langmuir-Freundlich, and Langmuir) were applied to the adsorption data to evaluate the adsorption phenomena. To this end, adsorption coefficients describing the adsorption characteristics of the studied compound were obtained. The results were demonstrated on one compound for each group of MPs (atenolol for beta-blockers, cyclophosphamide for cytostatics, benzotriazole for corrosion inhibitors, diclofenac for persistent compounds, and mecoprop (MCPP) for herbicides). These compounds were chosen for simplification and illustration.

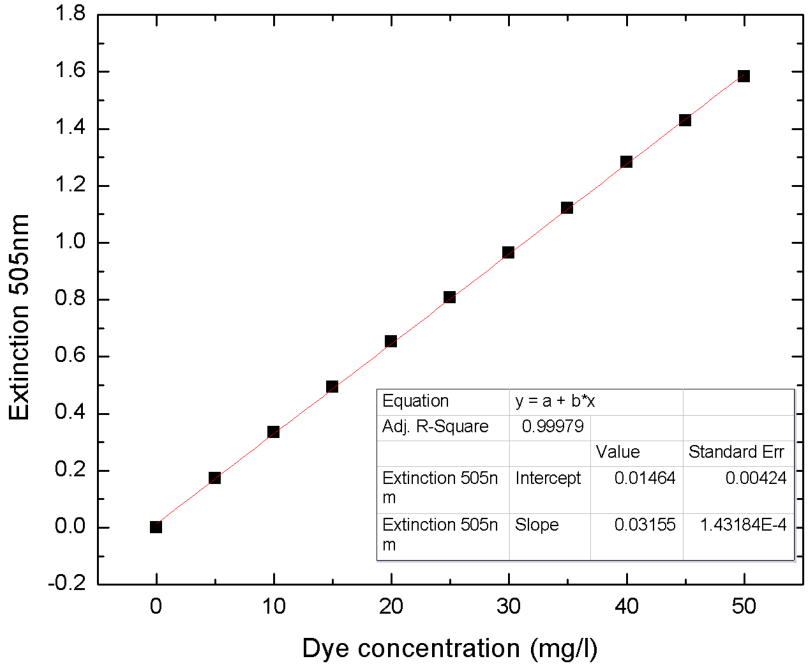
A two-pronged approach was chosen. In the first approach, adsorption kinetics with a single compound, allowed us to gain the perception of the adsorption capacity of studied substrates compared to conventionally used substrates (granular activated carbons). This knowledge was then completed by the implementation of the substrates in packed-bed columns, which treated a mixture of 27 MPs in tap water for 10 months. The combination of these two approaches gave a complex overview of the role of the adsorption process in the removal of MPs with help of CWs from wastewater and contribute to the comprehensive characterization of the substrates studied. The significance of this research lies also in the fact that adsorption of MPs on different soil media in packed-bed columns in continuous mode can be used as a single wastewater treatment step [258,259].

6.2 Methodology and materials

6.2.1 Experiments

6.2.1.1 Adsorption kinetics (batch experiments)

The selected substrates were: 100% sand (A), 85% sand and 15% activated (C) and non-activated biochar, 85% sand and 15% zeolite (E) purchased at PalaTerra, Hengstbacherhof/Germany. As a complementary method used for determining the adsorption capacity of selected substrates, the adsorption kinetics experiment with one compound as a proxy (dye Basovit red 400E, BASF) as an adsorbate was carried out. Experiments were performed in brown glass bottles, to avoid any possible photodegradation on a horizontal shaking table (Thermo Scientific) with a shaking intensity of 120 RPM. In all kinetic experiments, the amount of adsorbent was added as a suspension in the solution containing adsorbate at a known concentration (50 mg/l). The dye concentration in the soluble fraction was measured spectrometrically (DR 3900 VIS Spectral Photometer with RFID* technology, Hach Lange) with extinction determination. A calibration curve ($R^2 = 0,99979$) is presented in Fig. 6.1 which clearly shows that the concentration and the extinction are fitting to linear regression, a condition necessary to fulfill the Beer-Lambert law. Within the relation given it is possible to calculate the remaining dye concentration in the liquid solution and consequently the amount of adsorbed dye. The soils used for the adsorption experiments were sterilized by heating up to 105 °C for 6 hours to avoid interference of microorganisms in the adsorption process. The soils were also sieved in a sieving tower allowing just particles bigger than 0.180 mm to be present in the final mixture and so avoiding the presence of dust. Sieved soils were then stored in a cold, closed dark place. To compare studied sand-based substrates with well-known materials and to evaluate the reliability of the method, experiments with GAC (CarboTech AC GmbH) and RAC (NRS Carbon 0.5-2.5 type, Cabot Norit Nederland B.V.) were carried out. The physical properties of these two substrates are available in the supplementary data (Tab. S.2.1.-2.).



Equation	y = a + b*x		
Adj. R-Square	0.99979		
		Value	Standard Err
Extinction 505nm	Intercept	0.01464	0.00424
Extinction 505nm	Slope	0.03155	1.43184E-4

Fig. 6.1: Calibration curve of Basovit redE with conc. 50 mg/l.

In the first experiment, the selected amount of GAC, as well as RAC, is 1 g/l [260] which is left as a suspension in the solution. The 2 ml samples were taken in 20-minute intervals at the beginning of the experiment, presuming that the adsorption would be most rapid at the beginning. After ca. 2 hours of the experiment, the sampling period was prolonged to hourly units. The experiment was ended after the complete adsorption of the dye and reaching equilibrium. The samples were filtered through 0.45 μm filter and the absorbance was measured in the photospectrometer at the characteristic extinction value of the dye solution at 505 nm.

Next, the selected substrates A, C, E, and sand + 15 % non-activated biochar were studied. The non-activated biochar was included to establish the difference in terms of adsorption capacity between activated and non-activated biochar. The concentration of these substrates is 50 g/l. Such a high concentration of the substrates was chosen based on the measured adsorption capacity of the substrates during pre-experiments. As the adsorption rate was not as high as for GAC and RAC, the samples were taken on a daily, later weekly basis, in order to reach the equilibrium. To confirm the sterilization process and thus to exclude the biological potential of activated and non-activated biochar, experiments with the addition of specific nutrients were carried out. It was supposed that the bacteria, which could be present in the activated biochar, could be nourished by sources of nitrogen and carbon. With the addition of nutrients followed by the growth of bacteria, the elimination of the dye from the solution could be enhanced, because the dye is considered a potential source of carbon. In this kind of experiment, two types of nutrients (Carl Roth) were used, the first one is the Hoagland solution (components for preparation are showed in Tab. 6.1), used in our phytoremediation experiment [228]. As Tab. 6.1 shows, other inorganic compounds were also present in the solution, which could be consumed by the bacteria as nourishment. The second type was a mixture of nutrients used for the formulation of synthetic wastewater according to the standard OECD 303A (chemical components are showed in Tab. 6.2), which was used in our previous project EmiSûre. Concentrations of the used chemicals are available in supplementary data (Tab. S.1.1.-2.). The concentration of the dye and the substrates remained the same (50 mg/l and 50 g/l respectively). The 2 ml samples were taken each couple of hours, as it was presumed that the adsorption on these substrates is not very rapid. The experiments were terminated after 650 hours.

Tab. 6.1. Compounds for the preparation of the Hoagland solution.

Compound	**CAS number**
KH_2PO_4	7778-77-0
KNO_3	7757-79-1
$CaCl_2$ $2H_2O$	10035-04-8
$Mg(SO_4)$ $7H_2O$	10034-99-8
$NaHCO_3$	144-55-8

Tab. 6.2. Compounds for the preparation of the synthetic wastewater.

Compound	**CAS number**
Peptone	91079-38-8
Meat extract	68990-09-0
Urea	57-13-6
Potassium dihydrogen phosphate	7778-77-0

Lastly, the pure kinetic experiment of the substrates (50 g/l) and the dye (50 mg/l) was performed. The goal of this experiment was to reach an adsorption equilibrium and, based on the equilibrium data, determine adsorption kinetic orders and isotherm models.

6.2.1.2 Packed-bed columns

The soils were packed in 3 identical plexiglass columns (height of the column 1 m, inner diameter 0.04 m, Europlex), resulting in a height of a soil bed 0.8 m and height of a gravel bed 0.08 m used as drainage. The scheme of the installation is illustrated in Fig. 6.2.

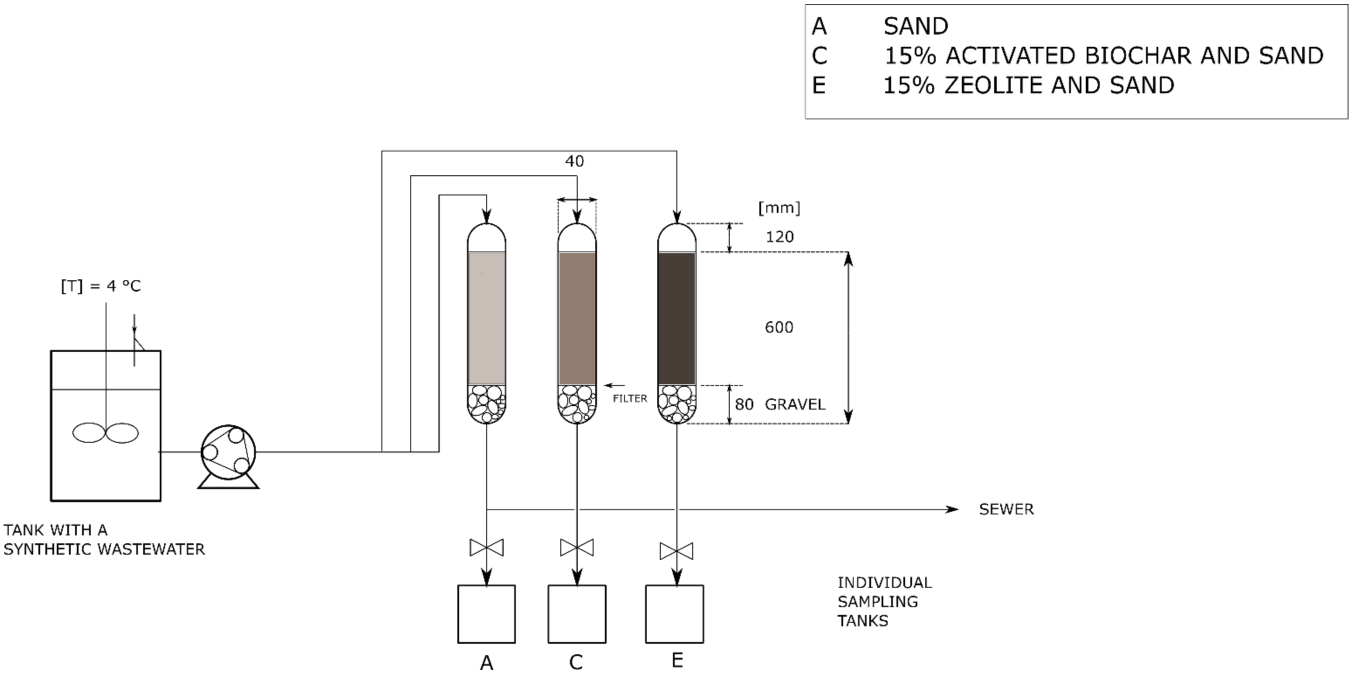


Fig. 6.2: Packed-bed adsorption columns installation.

The columns were wrapped in aluminum foil to exclude the contribution of photodegradation. Next, the columns were fed from the top to the bottom with a mixture of MPs in tap water with the help of a peristaltic pump (type 530S/R, provided by Watson-Marlow NV) whereas the influent mixture was stored in a cooling tank (4 °C, continuous stirring, Lely Center). The tap water was spiked with the mixture of 27 selected MPs (Tab. 6.3) in the range of concentrations 1 – 5 µg/l (Techlab, purity > 99.99 %). The comprehensive list presented in Tab. 6.3 relates the use of each compound with the selection criteria, identifying those of interest because of their legal obligation (i.e. clarithromycin and ciprofloxacin), consumption (i.e. atenolol), recalcitrant aspect (i.e. carbamazepine) or toxicity potential (i.e. fluorosurfactants).

Tab. 6.3: Selected MPs.

Application	Compound	CAS number	Therapeutic Group/Use	Selection criteria
Pharmaceuticals and metabolites	Atenolol	29122-68-7	Beta Blocker	Highly prescribed.
	Bezafibrate	41859-67-0	Lipid regulator	Highly biodegradable.
	Carbamazepine	298-46-4	Psychiatric drug	Mainly excreted as a hydroxylated metabolite. Control compound.
	Clarithromycin	81103-11-9	Antibiotic	Present in the Watch List (EU) 2015/495 of 20 March 2015 [261]
	Ciprofloxacin	85721-33-1	Antibiotic	Present in the Watch List (EU) 2018/840
	Cyclophosphamide	50-18-0	Cytostatic	High eco-toxicity impact.
	Diclofenac	15307-86-5	Analgesic/anti-inflammatories	Present in Directive 2013/39/EU.
	Erythromycin A	114-07-8	Antibiotic	Present in the Watch List (EU) 2015/495 of 20 March 2015
	Ketoprofen	22071-15-4	Analgesic/anti-inflammatories	Highly prescribed. Found in surface waters.
	Lidocaine	137-58-6	Anaesthetic	Highly prescribed. Found in surface waters.
	Metoprolol	51384-51-1	Beta Blocker	Highly prescribed.
	Propranolol	525-66-6	Beta Blocker	Highly prescribed. Found in concentrations above EQS.
	N4-acetylsulfamethoxazole	21312-10-7	Metabolite of Sulfamethoxazole	For mass balance.
	Sulfamethoxazole	723-46-6	Antibiotic	Old antibiotic, still in use. Scientific data are available.
Pesticides/Herbicides	Carbendazim	10605-21-7	Fungicide	Very persistent.
	DEET	134-62-3	Insect repellent	Very persistent.
	Diuron	330-54-1	Herbicide	Present in Directive 2018/105/E
	Isoproturon	34123-59-6	Herbicide	Present in Directive 2018/105/E
	Terbutryn	886-50-0	Herbicide	Very persistent.
	Mecoprop (MCPP)	7085-19-0	Herbicide	Found in surface waters.
	Tolyltriazole	29385-43-1	Fertilizer	Highly used. Most abundant in WWTPs discharging in the Sûre river.
	Glyphosate	1071-83-6	Herbicide	Under discussion.
	Aminomethylphosphonic acid (AMPA)	1066-51-9	Degradation product	Under discussion.
Fluorosurfactants	Perfluorooctanesulfonic acid (PFOS)	1763-23-1	Surfactant	Priority compound.
	Perfluorooctanoic acid (PFOA)	335-67-1	Surfactant	Of political concern.
Corrosion inhibitor	Benzotriazole	95-14-7	Corrosion inhibitor/Antiviral	Highly used. Most abundant in WWTPs discharging in the Sûre river.
Flame retardant	Tris(2-chloroisopropyl)phosphate (TCPP)	13674-84-5	Flame retardant	Highly used. Most abundant in WWTPs discharging in the Sûre river.

The first idea had been to operate the installation under continuous flow to see how this operation would influence the adsorption capacity of the studied substrates. However, the infiltration capacity decreased under continuous flow, therefore it was required to switch to an intermittent operation. This step was advantageous in terms of the hydraulic conditions. The infiltration conditions became steady with a watering cycle of 15 ml/min 3 times per day, where one cycle had a 30 min. duration. After passing the columns, the mixture was then led to sampling tanks, from which the overflow was connected to the sewer. The columns were provided with the overflow configuration in case the system became clogged. The columns were operated under the conditions described for 10 months. Despite the adapted conditions, a regular backwash of columns A and C (2-3x per week) was needed. However, regular backwashing helped to regulate the thickness of generated biofilm, therefore it helped to avoid possibly occurring bioremediative processes [262]. Samples were taken once per month and an analysis of nutrient content (Hach Lange cuvette text box), oxidation-reduction potential, conductivity, pH, and temperature was performed in-house (multi-portable parameter meters by Xylem Analytics Germany Sales GmbH & Co. KG). The samples were filtered with a 0.45 µm filter, frozen and prepared for the external analysis of MPs content.

6.2.1.3 Analytical methods

Concentrations of macronutrients in the adsorption kinetics batch, as well as in the packed-columns samples were measured in-house. For the kinetic, COD and TN were frequently measured, for the packed-bed columns, COD, TN, NO_3^- and NH_4^+ were measured. Further readings were taken for the pH value, the concentration of dissolved oxygen (DO), conductivity, temperature, and oxidation-reduction potential. These values are available in the supplementary data (section S.6). The samples of the MPs' content were filtered through a 0.45 µm filter and frozen at -20 °C. The concentration of MPs was analyzed externally at the Luxembourg Institute of Science and Technology (LIST). The MP samples were re-filtered and diluted with water/methanol 90/10, as preconditioning was not needed and then immediately analyzed by Liquid Chromatography (1260 Series, Agilent, Santa Clara USA) coupled to triple-quadrupole Mass Spectrometry (QTRAP 4500, AB Sciex, Framingham USA) according to the procedure described previously (Brunhoferova et al., 2021).

6.2.2 Calculations

6.2.2.1 Adsorption kinetics

For the kinetic experiments, rate constants were determined based on two established kinetic models; the pseudo-first-order was calculated from Eq. 6.1, where the integrated form of the equation is presented [263]:

$$\log(q_e - q_t) = \log\,(q_e - \left(\frac{k_1}{2.303}\right) t \tag{6.1}$$

where q_e and q_t are the adsorbate uptake at equilibrium and at the time t and k_1 is the pseudo-first-order rate constant. The pseudo-first-order rate constant can be obtained from the slope of the plot log ($q_e - q_t$) vs. t.

The pseudo-second-order was calculated from Eq. 6.2:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right) t \tag{6.2}$$

From the plot t/q_t vs. t the pseudo-second-order rate constant, k_2. can be estimated.

The adsorbed amount of dye per gram of adsorbent was calculated from Eq. 6.3-4:

$$q_e = \frac{V(C_0 - C_e)}{M} \tag{6.3}$$

for batch experiments [264] and

$$q_e = \frac{(C_0 - C_e)}{M} \tag{6.4}$$

for packed-bed experiments [242], where q_e amount adsorbed of dye per gram of adsorbent, V is the volume of the solution, M is the amount of the adsorbent used and c_0 and c_e are the concentrations of the dye in the initial solution and at equilibrium.

The removal rate was calculated from Eq. 6.5:

$$R.r.(\%) = \frac{c_0 - c_e}{c_0} * 100\ \% \tag{6.5}$$

6.2.2.2 *Packed-bed columns*

For the optimal conditions of the adsorption in packed-bed columns, empty-bed contact time (EBCT) was calculated (Eq. 6.6). As the typical value for the EBCT is 30 min, the parameters have been chosen in a way to come as close as possible to this value (in this case EBCT = 33 min).

$$EBCT = \frac{V_B}{Q_B} \tag{6.6}$$

where V_B is the volume of the bed and Q_B is the flow rate coming into the bed.

In order to describe the elimination of the MPs by adsorption, three commonly used models of adsorption under stable pH [265] (Langmuir, Langmuir-Freundlich and Freundlich) were created (Eq. 6.7-9)

$$q_e = K_F T^{1/nF} \tag{6.7}$$

$$q_e = q_m K_{LF}(T^n)/(1 + K_{LF}(T^n)) \tag{6.8}$$

$$q_e = (q_m K_L T)/(1 + K_L T) \tag{6.9}$$

where q_e is the amount of adsorbate adsorbed on the adsorbent at equilibrium, q_m is the maximum adsorption capacity according to Langmuir and Langmuir-Freundlich models, T is reaction time, K_{LF} and K_L are parameters corresponding to adsorption intensity in Langmuir-Freundlich and Langmuir equations respectively, n is a constant corresponding to the heterogeneity of the adsorbent in Langmuir-Freundlich equation and K_F and n_F are constants defining adsorption capacity and intensity in the Freundlich model [266].

The height of the mass transfer zone (MTZ) is calculated according to Eq. 6.10:

$$Height\ MTZ = h_B * 1 - \frac{q_b}{q_s} \tag{6.10}$$

Where h_B is the height of the bed, q_b is the adsorbed amount in the break point and q_s is the adsorbed amount in the saturation point.

6.3 Results

6.3.1 Adsorption kinetics

Results of the comparative experiments among different types of activated carbon are showed in Fig.6.3, where the extinction is observed during a reaction time for a given amount of carbon and related to the removal of adsorbent from the liquid fraction. 1 g/l of GAC can adsorb the dye completely in 50 hours, whereas RAC requires 170 hours (Fig. 6.3a). Under the same conditions (concentration of the adsorbent and adsorbate) the substrate C - 15% activated biochar, which proved in the past to be the most effective one, showed adsorption of the dye to a maximum of 7 % over 400 hours (Fig. 6.3b). The kinetic profiles of these experiments are available in the supplementary data (Tab. S.5.1.-2.).

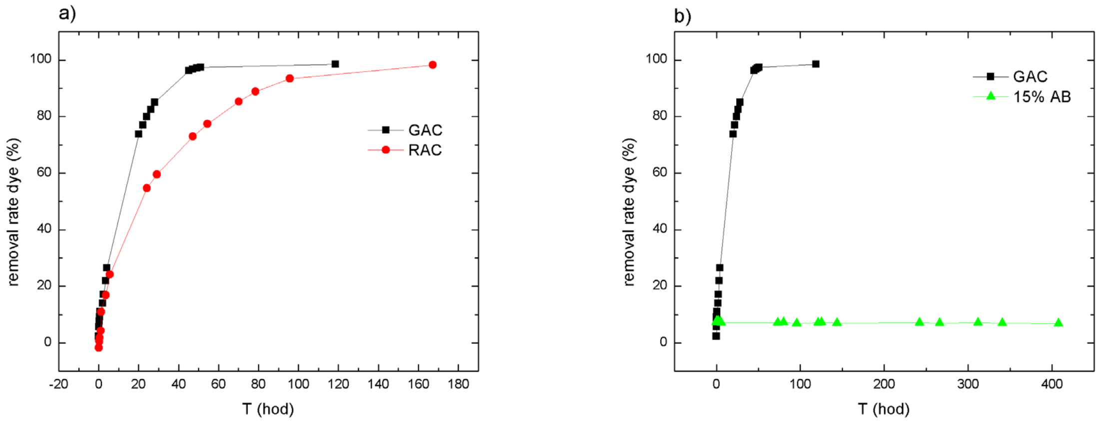


Fig. 6.3: Comparison of adsorption capacity of GAC and RAC (1 g/l), c dye 50 mg/l (a) and comparison of adsorption capacity of GAC and 15% activated biochar (1 g/l), c dye 50 mg/l (b).

Kinetic orders are determined for comparative experiments with GAC and RAC resulting in corresponding constants. Unfortunately, even with the high number of substrates studied: 50 g/l, it was not possible to reach the equilibrium and therefore to establish kinetic orders for the dye adsorption on the substrates. The values for k_1 and k_2 are calculated from the slope and intercept $\log(q_e\text{-}q_t)$ vs. t and t/q_t vs. t respectively applying the equations (6.1) to (6.4). The values gained are presented in Tab. 6.4.

Tab. 6.4. Kinetic constants for pseudo-first- and -second-order for substrates GAC and RAC.

adsorbent	**pseudo-first-order**			**pseudo-second-order**		
	k_1 (hod^{-1})	R^2	q_e (mg/g)	k_2 (g/mg hod^{-1})	R^2	q_e (mg/g)
GAC	0.00031	0.9854	52.747	0.00248	0.9839	53.476
RAC	6.57E-5	0.9732	43.171	0.00093	0.9799	54.644

For GAC, the q_e calculated from kinetic models was closer to the experimental value of q_e, therefore it is likely that this reaction took place in the pseudo-first-kinetic order. On the other hand, for RAC, the experimental q_e was closer to the one from the pseudo-second-kinetic order, therefore this reaction was probably pseudo-second-kinetic order [267,268].

The experiments for determining the microbial potential of biochars were carried out with activated and non-activated biochar in three different mixtures: a blank one (without any nutrients), in a nutrient mixture used previously in the lysimeter experiments and in a mixture containing Hoagland solution. These experiments did not confirm any presence of microbes, as no increased dye removal (Fig. 6.4a and b) was observed due to the presence of sources of C and N. However, decreasing concentrations of COD and TN could be observed, leading to the presumption that the nutrients are adsorbed in the adsorption centers as well. Based on these findings, it seems that the contribution of the microbial potential on the adsorption can be excluded and the appropriateness of the soil sterilization method is confirmed. The adsorption kinetics of the dye are shown in the following figures: activated biochar (c 50 g/l) a) and non-activated biochar (c 50 g/l) b).

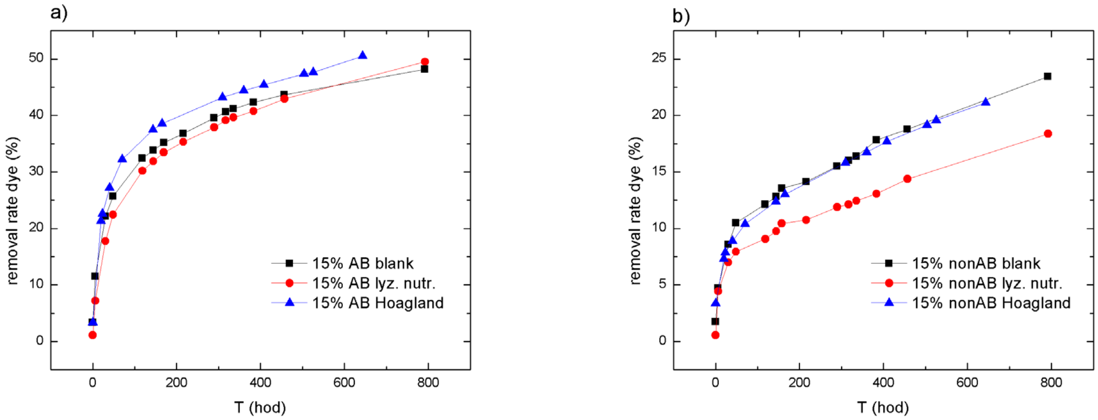


Fig. 6.4: Adsorption kinetics of the dye (c 50 mg/l) on 15% activated (a) and non-activated (b) biochar (c 50 g/l).

Another kinetic experiment indicated that the 15% non-activated biochar showed lower adsorption capacity even at much higher concentrations than 15% activated biochar: the non-activated biochar in concentration 50 g/l reaches 20 % removal of the dye, whereas the activated form in the same concentration removed about 50 % of the adsorbate. The 15 % activated biochar in 5x lower concentration than the one of 15 % non-activated biochar still surpassed the non-activated form in terms of the dye removal (removal of the dye 25 %). The comparison of adsorption efficiency of 15% activated biochar (10 and 50 g/l) and 15% non-activated biochar is shown in Fig. 6.5. These experiments demonstrate the better performance of the activated biochar over the non-activated.

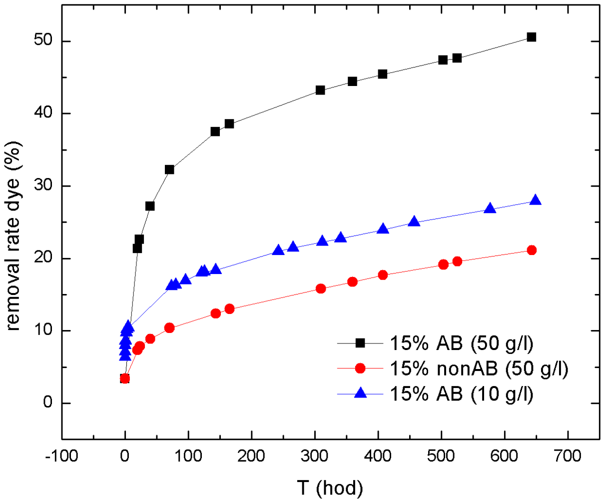


Fig. 6.5: Comparison of adsorption efficiency of 15% activated biochar (10 and 50 g/l) and 15% non-activated biochar, c dye 50 mg/l.

The kinetic experiments of studied substrates A, C and E confirm the previous hypothesis: that substrate C (15% AB) has the highest adsorption capacity. This fact is demonstrated by removal of the dye in a total of 84 %, followed by 15 % zeolite with 57 %, and finally by sand, reaching removal of 33 % in 234 days. The kinetic profile is shown in Fig. 6.6. Detailed concentration profiles and the removal rates are available in the supplementary data (Tab. S.5.3.-5.).

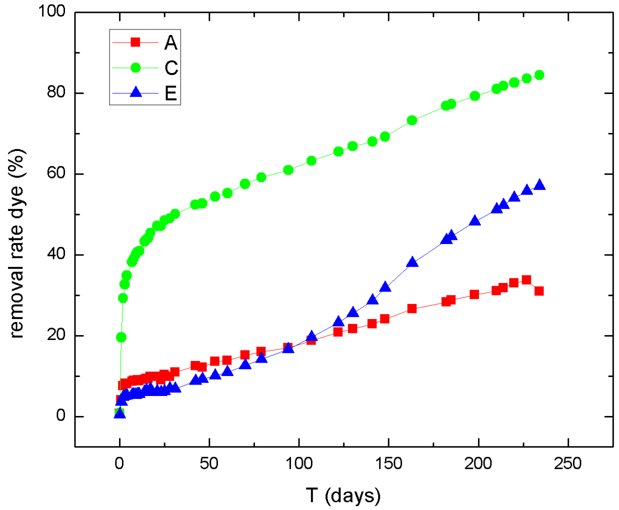


Fig. 6.6: Comparison of adsorption efficiency of studied sand-based substrates (50 g/l), c dye 50 mg/l.

6.3.2 Adsorption on packed-bed columns

6.3.2.1 General parameters

During the investigation, the operation mode was adapted from continuous to intermittent due to the perceiving clogging. This resulted to be advantageous, as the EBCT was prolonged and approached values typical for lab-scale constructed wetlands, which were recently investigated in our previous project (EmiSûre, Interreg Greater Region, 2017 - 2021). This allowed also better estimation of the adsorption's role within the wetlands and a better understanding about the role of unconventional sand-based substrates compared to common used activated carbon materials.

Stable results of effluent general parameters (e.g. pH, electrical conductivity) together with medium-high removal efficiencies of COD (on average 74 % for column A, 81 % for column C, and 79 % for column E) suggest that the system could be efficient for stable removal of MPs (Tab. 6.5).

Tab.6.5. Influent and effluent concentrations of selected parameters ± standard deviation (N = 15).

parameter (N = 15)	**influent**	**effluent A**	**effluent C**	**effluent E**
COD (mg/l)	76.3±15.8	33.7±9.7	19.6±6.1	32.9±7.9
EC (μS/cm)	252±17.5	408±51.4	364±44.7	395±47.4
pH (mg/l)	8.25±0.3	8±0.3	8.16±0.3	8.02±0.2
E (mV)	235±29.1	221±29.3	229±29.8	222±29.6

This fact is supported by results of the studied substrates' characterization reported in Tab.6, especially for parameters like specific surface area (BET) (A 0,7 m^2/g, E 7,7 m^2/g, and C 19,9 m^2/g) and median pore volume (smallest with 69.08 mm in case of C), which support our previous estimation about the preferred candidacy of substrate C for effective removal of MPs. Other characterization parameters such as bulk density, porosity and others are included in Tab. 6.6 while results from the analysis of mineral phase using X-ray are available in the supplementary data (Tab. S.4.).

Tab. 6.6. Results of characterization parameters of substrates A, C, and E.

Parameter	**substrate A**	**substrate C**	**substrate E**
BET (m^2/g)	0.65	19.89	7.74
pH	8.1	8.2	7.9
Bulk density by Helium pycnometer (g/cm^3)	2.73	2.62	2.59
Median pore diameter (Vol) (mm)	104.01	69.08	112.14
Bulk density by mercury intrusion porosimetry (g/cm^3)	0.65	0.68	0.83
Apparent (Skeletal) density (g/cm^3)	2.34	2.2	2.18
Porosity (Vol. %)	72.16	68.91	61.72
Cu (mg/kg)	83	84	60

6.3.2.2 Removal of micropollutants

The columns were operated under the previously mentioned conditions for 10 months. Medium-high removal efficiencies of COD (esp. in case of column C) and stable values of pH and electrical conductivity indicate stable conditions for the removal of MPs. In figure 6.7, on the example of atenolol, breakthrough of a compound according to the number of bed volumes and the volume itself is demonstrated. From this figure it is evident that the break point occurred after treatment of approx. 300 l of treated wastewater with the exhaustion after ca 600 l. From the adsorption capacity at break point (when the adsorbate leaves the column effluent [255]) and saturation point (further adsorption on the substrate is not possible), the height of the mass transfer zone, i.e. the area with highest mass transfer speed [269] was detected, after 37 cm of the bed height (calculated according to Eq. 6.10). These parameters are crucial for further scale-up of industrial packed-bed columns.

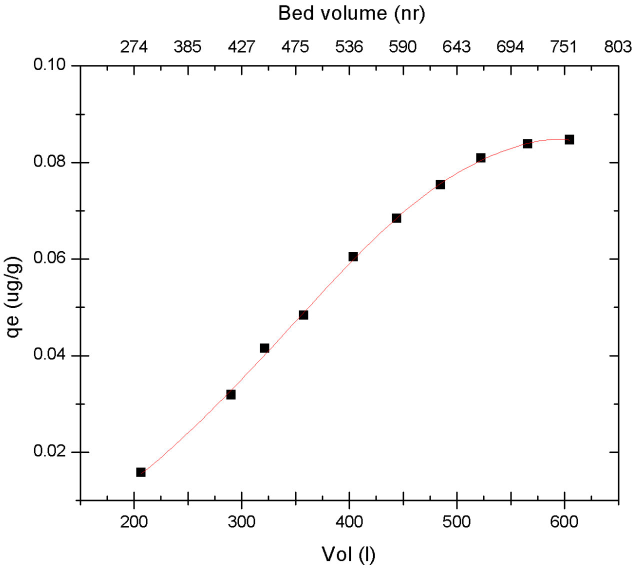


Fig. 6.7: Breakthrough curve of atenolol.

In the case of column C, 18 out of 27 compounds were removed on average with high efficiency (80 – 99 %) including compounds known to be well absorbable, e.g. beta-blockers [175]; antibiotics [270]; corrosion inhibitors [271]), but also persistent ones, e.g. diclofenac and lidocaine. Removal efficiency of column C for 27 targeted MPs is shown in Fig. 6.8. Some compounds, e.g. bezafibrate, carbamazepine, diuron, sulfamethoxazole, were removed with very high removal rates when exposed to vertical subsurface flow constructed wetlands (above 99 %). These compounds are known to be poorly biodegradable [2,262] and their successful removal through adsorption in our experiment confirms that they tend stronger to adsorption. Five compounds were removed on average with medium efficiency (40 - 80 %): AMPA, DEET, glyphosate, MCPP and N-acetyl sulfamethoxazole; followed by compounds removed poorly (0 – 40 %): ciprofloxacin, cyclophosphamide, PFOA, PFOS.

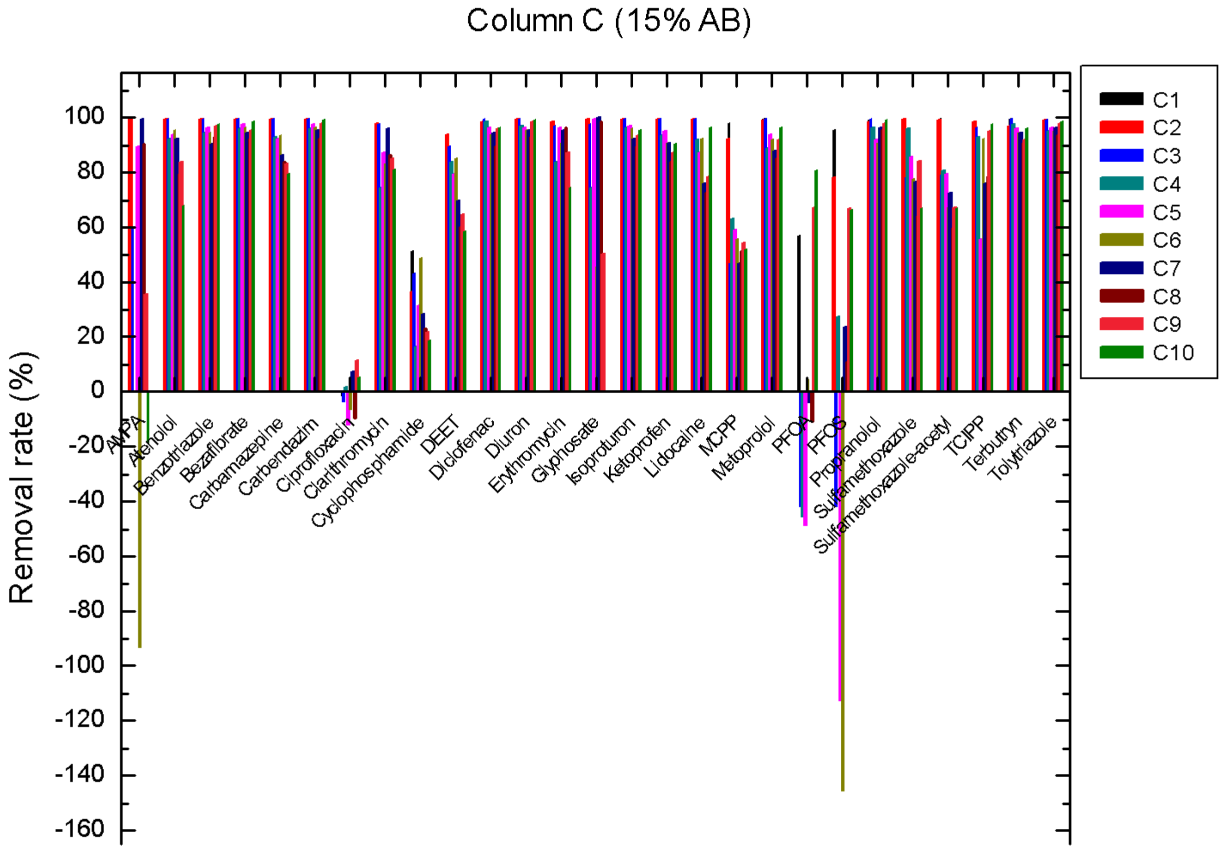


Fig. 6.8: Removal efficiency of column C for 27 targeted MPs, C1 – 10 are campaigns measured with an interval of one month.

In cases of PFOA and PFOS, negative removal is observed in all types of substrates, confirming evidence that per-fluoroalkyl substances are likely to be desorbed in sand-based materials [272] and released in the water effluent. In the case of ciprofloxacin, the analytical stability of this compound is rather poor in the influent, therefore to determine the effluent concentrations is complicated. Low and later negative elimination rates for AMPA are probably due to the fact that AMPA (a degradation product of glyphosate) tends to retransform back to its parent compound [238,239]. Cyclophosphamide, DEET and MCPP show decreasing elimination tendency with time, suggesting that these compounds are saturated onto the substrate. The concentration profiles of the studied compounds are available in the supplementary data (Tab. S.7.). Interestingly, the addition of zeolite does not improve adsorption efficiency of the sand-based material, even though BET surface area of the substrate E is almost 12x higher and zeolite belongs to persuasive adsorbent materials. This trend was observed during our previous experiments with sand-based constructed wetlands, where the addition of zeolite did not result in higher adsorption efficiency of the substrate. Also, columns with substrates A and C are operated under similar hydraulic conditions (regular backwash 2-3x per week), whereas columns with the zeolite substrate never showed any clogging during the operation, therefore no backwash was needed. Despite these facts, when the average removal rate is compared (Fig. 6.9), substrate C results, with an extensive lead, in being the most efficient adsorbent among substrates used for elimination of MPs from tap water. This is probably owing to the highest BET surface area and lowest median pore diameter, which could result in better retention of the MPs in the substrate [273,274].

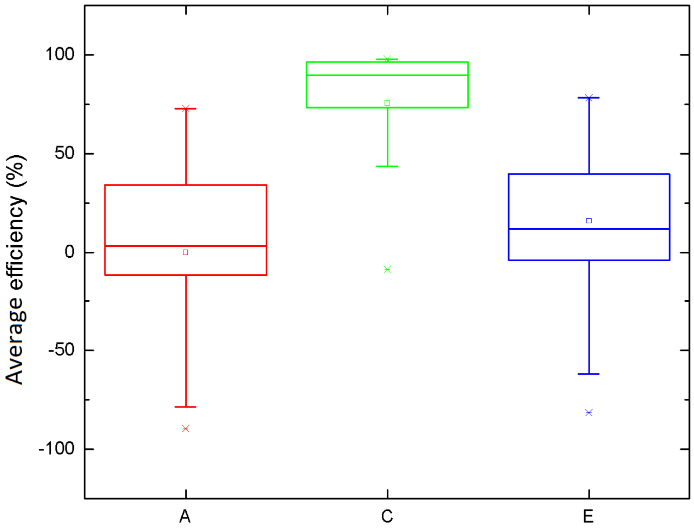


Fig. 6.9: Comparison of average removal rates of the studied substrates considering MPs removal from tap water.

6.3.2.3 Adsorption isotherms

For almost all of the compounds adsorbed on the substrate C, models of three adsorption isotherms (Freundlich, Langmuir-Freundlich and Langmuir) were created. The models were not created for AMPA, ciprofloxacin, glyphosate, PFOA, PFOS and Sulfamethoxazole-Acetyl because of concentration biases caused by analytical uncertainties, desorption events and occurring retransformation of some of the compounds. Table 6.7 shows adsorption parameters calculated according to equations (6.7) to (6.9) for a representative key member from the groups studied: atenolol for beta-blockers, cyclophosphamide for cytostatic, benzotriazole for corrosion inhibitors, diclofenac for persistent compounds and MCPP for herbicides. The parameters for all other compounds are shown in the supplementary data (Tab. S.8.).

Tab. 6.7. Adsorption coefficients of Freundlich, Langmuir-Freundlich and Langmuir isotherms for stated compounds.

Compound	Freundlich			Langmuir-Freundlich				Langmuir		
	K_F	n_F	R^{2a}	q_m*	K_{LF}	n	R^2	q_m*	K_L	R^2
atenolol	0.0014	1.3572	0.9313	0.10	8.9E-05	1.9416	0.9752	0.22	0.00234	0.9507
cyclophosphamide	6.6E-04	1.3508	0.9121	0.047	3.9E-05	2.1335	0.9633	0.11	0.0023	0.9325
benzotriazole	5.0E-04	1.0518	0.9561	0.14	7.0E-05	1.8573	0.9740	0.9	4.6E-04	0.9591
diclofenac	2.1E-04	0.9668	0.9650	0.15	2.1E-04	1.4693	0.9653	0.17	1.5E-06	0.9643
MCPP	2.2E-04	1.3293	0.9671	0.025	8.7E-04	1.3315	0.9752	0.41	0.0021	0.9748

*μg/g

In most of the cases, the adsorption corresponds to the Langmuir-Freundlich model, except for the antibiotics. These compounds normally fit better into the Langmuir model, e.g. in the case of sulfamethoxazole [181] or ciprofloxacin [270]. The Freundlich isotherm is empirical and it is suitable for multi-layer adsorption, whereas the Langmuir isotherm is a theoretical construct and accords better with monolayer adsorption [268,275]. A combination of both constitutes results in the Langmuir-Freundlich model which considers the heterogeneity of the solid surface and was the most suitable model for the removal of emerging contaminants in previous studies [186]. This is also the case within this study, due to several factors: 1. the values of the modelled q_m parameter are closest to the experimental values of q_e of the demonstrated compounds [276]; 2. the correlation factor R^2 is the highest for the Langmuir-Freundlich model [263]; and 3. the values of the intensity parameter n are highest in case of Langmuir-Freundlich isotherm model, suggesting that the adsorption described with this model is the strongest [277]. Fig. 6.10 presents experimental adsorption data of atenolol and cyclophosphamide and their comparison with the previously mentioned adsorption models. The best fit of the Langmuir-Freundlich model to the experimental data in both cases confirms the discussion above. The results described help to complete the characterization of these unconventional substrates, as their adsorption capacities are substantially lower (tenths of μg/g) than adsorption capacities of other non-carbon homogenous materials, e.g. sand or zeolite, in units of μg/g [278], or well-known sorbents, e.g. activated carbons, in mg/g.

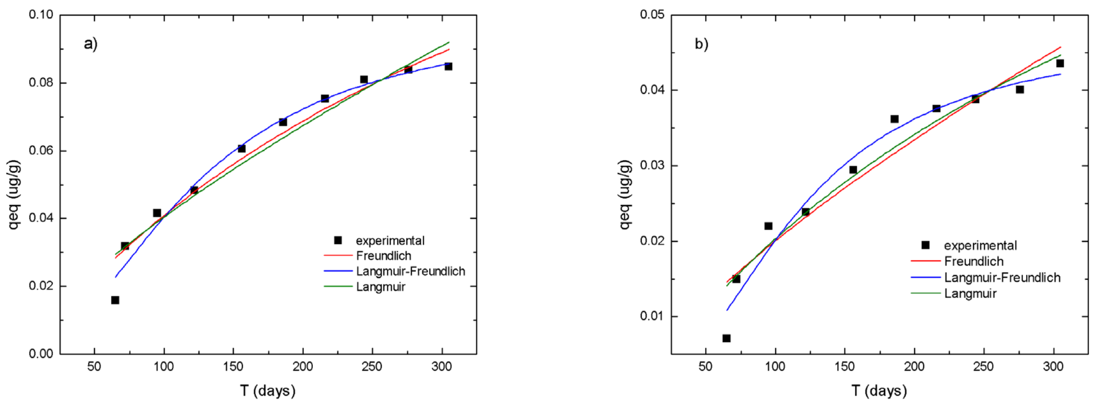


Fig. 6.10: Comparison of experimental adsorption data and Freundlich, Langmuir-Freundlich and Langmuir models for atenolol (a)) and cyclophosphamide (b)).

6.4 Conclusions

The present study has compared the adsorption capacity of the unconventional sand-based substrates previously used in pilot scale vertical flow subsurface constructed wetlands (EmiSûre, Interreg Greater Region 2017-2021) and the well-known sorbents (granulated activated carbons). The main results can be summarized as follows:

- Results of one-compound adsorption with dye as a proxy lead to the conclusion that the studied substrates have substantially lower capacity than conventionally used granulated activated carbons.
- Experiments for determining the microbial potential were carried out on substrates with admixture either with activated or non-activated biochar, resulting in excluding the microbes' activity and additionally leading to an understanding that the activated biochar is more efficient in its adsorption capacity than the non-activated one.
- Among all the substrates studied, the single-compound kinetic experiments showed that the activated biochar is the most efficient substrate, followed by zeolite admixture and pure sand.
- This fact was confirmed by the results from packed-bed column experiments, where the admixture with activated biochar proved to be the most capable in terms of removal of 27 MPs compared to

the zeolite admixture and pure sand. Additionally this mixture showed almost no desorption during the whole operation of 10 months in contrast to the other two substrates.

- A broad characterization of the physical and chemical properties of the studied substrates has been carried out, resulting in the fact that the highest efficiency of the activated biochar is probably caused due to its broad BET surface area and small median pore diameter.
- For the 27 MPs, adsorption models were created, and the best fitting model was evaluated for its favorable fit to experimental data and other parameters discussed above; the best model for most of the MPs is the combined Langmuir-Freundlich. This model allows a description of optimal adsorption characteristics of the substrates studied, such as maximum adsorbed capacity, heterogeneity of the surface of the substrate, which could eventually help to establish the lifetime or to design a tailored treatment.
- The parameters needed for industrial upscale of the sand-based substrate with activated biochar were specified, such as height of mass transfer zone, breakthrough and saturation point related to treated synthetic wastewater and number of bed volumes.

With the knowledge obtained from this study, we believe the characterization of the unconventional sand-based substrates used contributes significantly to understanding and consequent successful application of these substrates, especially the sand-based substrate with admixture of activated biochar, in nature-based solutions in a broad scope.

Acknowledgement

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Supplementary data

Supplementary data to this article can be found online.

7 Publication III - Removal of 27 micropollutants by selected wetland macrophytes in hydroponic conditions

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Abstract

In this work, the primary focus is given on a mixture of 27 micropollutants (pharmaceuticals, pesticides, herbicides, fungicides and others) and its removal from aqueous solution by phytoremediation. Phytoremediation belongs to technologies, which are contributing on removal of micropollutants from wastewater in constructed wetlands. Constructed wetlands can be used as an additional step for elimination of micropollutants from municipal medium-sized wastewater treatment plants. To our knowledge, such a broad variety of micropollutants was never targeted for removal by phytoremediation before. In this work, we carry out experiments with 3 emergent macrophytes: *Phragmites australis*, *Iris pseudacorus* and *Lythrum salicaria* in hydroponic conditions. The selected plants are exposed to mixture of micropollutants in concentrations 1-14 mg/l for a time period of 30 days. The highest affinity for phytoremediation is detected at groups of fluorosurfactants (removal rate up to 30 %), beta-blockers (removal rate up to 50 %) and antibiotics (removal rate up to 90 %). The leading capability for micropollutant uptake is detected at *Lythrum salicaria*, where 25 out of 27 compounds are removed with more than 20 % efficiency. The results demonstrate well usefulness of this technology e.g. in an additional treatment step, because the mentioned groups of micropollutants are removed with comparable or even higher effectivity, than it is in case of conventional wastewater treatment plants.

Keywords: micropollutant removal; emergent macrophytes; hydroponic conditions.

7.1 Introduction

The use of pharmaceuticals, herbicides, pesticides and other compounds has been increasing in the past decades due to industrialization, agriculture, increased life expectations, etc. This trend creates many technological challenges, as these compounds, so-called micropollutants (MPs) or emerging contaminants, are poorly removed by conventional wastewater treatment plants (WWTPs) and thus may enter the aquatic environment [1,119,250,279,280] in relevant concentrations. The presence of MPs in water bodies creates also environmental challenge, as it may lead to negative effects, including spread of antibiotic resistance, short-term and long-term toxicity [281,282] and thus also possible impacts on human health [10]. Nowadays no legal limits regarding MPs in WWTPs effluents have been defined. However, the European Commission has been especially receptive to the topic of MPs contamination and released an updated Watch list [16] with 15 emerging contaminants, including macrolides and hormones. As this list obliges EU member states to monitor emerging contaminants, more European countries started to anticipate future decisions with national and local requirements. As example, the Swiss government identified 100 out of the 700 WWTPs that will be supported with an additional treatment step in order to achieve an average of 80 % removal for selected MPs [213]. The upgrade of existing WWTPs with an additional step is considered necessary, so that the presence of MPs in aquatic environment is avoided.

Generally common advanced technologies, such as activated carbon adsorption, advanced oxidation processes and membrane separation are extensively investigated [102]. Unfortunately, these processes have cost-prohibitive background. Constructed wetlands (CWs) are a promising technology especially for treatment of effluent of small and medium-sized WWTPs [127,255,283], which is the case for example of the Greater Region (Luxembourg, Wallonia, Rhineland- Palatinate, Saarland, Lorraine). Due to their low cost availability, easy set up and good performance [119,256,283], CWs have been recently exploited in the Interreg Greater Region project 'EmiSûre' (N 013-2-03-049) [284–286] with a planted vertical subsurface flow configuration. The project led to satisfying results in terms of global removals, however, individual contribution of removal technologies (i.e. sorption, biodegradation, phytoremediation) in CWs was not addressed. Therefore, a methodology has been set up to better quantify the contribution of each elimination mechanism minimizing the cross effect of the other. Particularly, current research intends to determine the mechanism for removal of MPs by specific type of plants, so-called phytoremediation.

Phytoremediation is a clean-up technology, where plants are able to remove pollutants from the environment [287–289]. It is composed from several processes, such as rhizofiltration, phytovolatilization or phytodegradation [290,291]. We suppose that abundant mechanisms in this case could be phytodegradation, as it is most suited for organic chemicals with log $K_{\mathrm{OW}} = 0.5 - 3.0$ [292] or phytovolatilization, which suits for low-molecular-weight compounds [184].

27 compounds are selected based on different criteria: those known to be highly excreted (in the case of pharmaceuticals) or with potential eco-toxicity (i.e. cytostatics), but also those to be under observation in line with the European Commission strategy and KomS guidelines [293]. To the best of our knowledge, removal of such a wide spectrum of MPs from liquid solution by specific plants has never been targeted before. Three types of emergent macrophytes, *Phragmites australis* (common reed), *Iris pseudacorus* (yellow flag) and *Lythrum salicaria* (purple loosestrife) are investigated. *Phragmites australis* belongs to the most used plants in CWs thanks to its extensive root and rhizome system [294], and together with *Iris pseudacorus* and *Lythrum salicaria* it was used in the EmiSûre project at vertical subsurface flow configuration of CWs. In case of *Phragmites* and *Iris* the advantage of combining long with short roots is considered beneficial as it is expected to increase the uptake of MPs in CWs configuration. *Lythrum* is known for its ability to build a subterranean system of roots [295] with extensive colonization of arbuscular mycorrhizal fungi (AMF) [296]. Symbiotic relationship between plants and AMF increases the ability of the plant to uptake nutrients and pollutants from different media [229,297–299]. To establish the potential for MPs to be up taken by selected wetland plants, experiments in so-called hydroponic conditions [300] are carried out.

Hydroponics as technology for plant growth is recently gaining increasing attention, as soil has been identified as a limiting factor for the plant growth [301,302]. It is used in many different areas, such as vertical farming, farms for cultivation of clean, healthy and fresh vegetables, but also in urban environment for reduction of water needs, environmental cultivation and others [301]. Usage of *Phragmites australis* in hydroponics is possible e.g. for gravel beds and treatment of wastewater, which have been already successfully demonstrated [303] or for removal of personal care products in hydroponics [304]. The knowledge gained about usage of *Iris* in hydroponics can be in future utilized in removal of e.g. pesticides [305]. One of the goals of this work is to establish optimal conditions for hydroponics, as this technology has potential in many different areas. The plants are exposed to different conditions not just to optimize the uptake of MPs by the plants, but also the growing conditions themselves.

This work is a part of a PhD research, aiming to quantify mechanisms/technologies in CWs as additional step in terms of MPs' removal from municipal wastewater. It is evident, that the plants themselves do not have such a vigorous capacity regarding elimination of MPs, especially in case of 27 compounds, as CW as a whole unit [171,306]. Therefore, concentrations in ranges of mg/l are selected [111,307,308], to ensure that the removal by the plants could be measured, as the main aim of the PhD research is to quantify the removal mechanisms with respect to the selected media's ability, not directly to its application to the real conditions.

The research presented in this publication aims: 1. to evaluate the potential of the selected macrophytes to remove studied MPs, 2. to assess the differences of adapted and fresh plants on the removal of MPs, 3. to appraise the role of phytoremediation in the removal mechanisms/technologies of CWs.

7.2 Methodology

7.2.1 Components and chemical compounds

One-liter jars with lid and chemicals needed for preparation of nutrient solution (so-called Hoagland solution (Smart & Barko, 1985)) are purchased at Carl Roth GmbH & Co. Kg. Three wetland plant species, *Phragmites australis*, *Iris pseudacorus* and *Lythrum salicaria* are from re-natur GmbH. A special LED lamp, providing artificial sunlight with 10 light intensities is provided by Lovebay International Limited. Portable meters for analyses of conductivity, oxidation-reduction potential, etc. are purchased at Xylem Analytics Germany Sales GmbH & Co. KG. For the measurement of the nutrients amount (nitrogen, phosphorus), the Hach Lange test cuvette box is used. 27 MPs compounds (Tab. 7.1) are all provided by Techlab, France (purity > 99.99 %). Compounds for the preparation of the nutrient solution are purchased at Carl Roth, Germany.

Tab. 7.1: Selected micropollutants.

Group	**Substance**	**CAS number**	**Therapeutic Group**	**log K_{ow}**
	Atenolol	29122-68-7	Beta Blocker	0.16
	Bezafibrate	41859-67-0	Lipid regulator	4.25
	Carbamazepine	298-46-4	Psychiatric drug	2.45
	Clarythromycin	81103-11-9	Antibiotic	3.16
Pharmaceuticals	Ciprofloxacin	85721-33-1	Antibiotic	0.28
	Cyclophosphamide	50-18-0	Cytostatic	0.63
	Diclofenac	15307-86-5	Analgesic/anti-inflammatories	4.51
	Erythromycin	114-07-8	Antibiotic	8.9

	Ketoprofen	22071-15-4	Analgesic/anti-inflammatories	3.12
	Lidocaine	137-58-6	Anesthetic	2.26
	Metoprolol	51384-51-1	Beta Blocker	1.88
	Propranolol	525-66-6	Beta Blocker	3.48
	N-acetyl sulfamethoxazole	21312-10-7	Metabolite of Sulfamethoxazole	0.86
	Sulfamethoxazole	723-46-6	Antibiotic	0.89
Corrosion inhibitor	Benzotriazole	95-14-7	Corrosion inhibitor/Antiviral	1.44
Pesticides/Herbicides etc.	Carbendazim	10605-21-7	Fungicide	1.52
	Deet	134-62-3	Insect repellents	2.02
	Diuron	330-54-1	Herbicide	2.68
	Isoproturon	34123-59-6	Herbicide	2.87
	Terbutryn	886-50-0	Herbicide	3.74
	Mecoprop	7085-19-0	Herbicide	3.13
	Tris(2-chloroisopropyl)phosphate	13674-84-5	Flame retardant	2.59
	Tolyltriazole	29385-43-1	Fertilizer	1.08
	Glyphosate	1071-83-6	Herbicide	-3.4
	AMPA (Aminomethylphosphonic acid)	1066-51-9	Degradation product	-1.63
Fluorosurfactants	Perfluorooctanesulfonic acid (PFOS)	1763-23-1	Surfactant	4.49

	Perfluorooctanic acid (PFOA)	335-67-1	Surfactant	4.81

7.2.2 Plants used for the experiment

The plants are chosen based on their usage in CWs, which are installed as a polishing step of effluents from 2 WWTPs in Luxembourg (WWTPs Reisdorf and Echternach [285], 4000 PE and 36000 PE respectively). In the first part, some of the controls are used like 'fresh', meaning that they did not get in contact with any wastewater effluent before and they are received directly from the producer (*Phragmites, Iris, Lythrum* fresh, controls A, B, C). Other reference plants, *Phragmites* and *Iris*, are from Reisdorf WWTPs, where they were exposed to wastewater effluent for 16 months (labeled as *Phragmites, Iris* adapted, controls D and E). These plants are chosen to evaluate the impact of long-term plant exposition to nutrients and MPs from wastewater effluent. The controls used in the experiment are stated in Tab. 7.2.

Tab. 7.2: Controls used in the experiment.

Label of a control	A	B	C	D	E
Type of a plant	*Phragmites* fresh	*Iris* fresh	*Lythrum* fresh	*Phragmites* adapted	*Iris* adapted

7.2.3 Conditioning phase

Prior the experiment, a conditioning phase has been considered necessary. For this phase, the soil is removed from the plants to avoid the soaking up of the MPs on the soil and the roots are immersed for 1 min to MICROPUR MC 1T medium [280] to inhibit the microbial activity and therefore to exclude the biodegradation and sorption of studied MPs. The antimicrobial medium is removed by rinsing of the roots for 30 s with Milli-Q water. Hoagland solution is prepared, to ensure a proper nutrition environment for the plant growth in hydroponic conditions. As this solution is designed for plants growing from sediment, where nitrogen and phosphorus source is provided from the substrate, the adaption is needed by adding source of these two missed elements. In the first part of the phytoremediation experiment, $NaNO_3$ and KH_2PO_4 are used as a source of nitrogen and phosphorus.

7.2.4 Phytoremediation experiment

After conditioning for one week, the plants are removed from the solution and dead biomass is eliminated. Plants are weighted 4 times: 1) before conditioning phase, 2) after conditioning phase, 3) before removal of dead biomass and 4) after removal of dead biomass. Next, fresh Hoagland solution is prepared and stock solutions of 27 MPs in concentrations 1 – 14 mg/l [184,305,307,309] in methanol are added (4.25 % solution). The jars are then filled to 1 l volume with Milli-Q water, covered with aluminium foil and closed with polystyrene lid, to exclude photodegradation. After 10 days of experiment in presence of MPs some phytomass of the plants turned into necromass (dead phytomass [310] indicating a loss in physiological activity. This is also confirmed with increasing concentrations of soluble nutrients in the growing solution [311–313].

Therefore, the experiments are repeated under following optimized conditions:

- adaptation of Hoagland solution: use of KNO_3 instead of $NaNO_3$, since Na is toxic to plants in concentrations higher than 50 mg/l [314];
- prolonged time of conditioning phase (2 weeks instead of 1 week);
- usage of LED lamp providing artificial sunlight, ensuring optimal growing conditions for plants [315];

- usage of aluminium ‘roof’ instead of polystyrene lid, ensuring oxygen input in the studied environment [315].

The second phase of the experiment is proceeded as described previously, with mentioned modifications on the methodology. The inorganic salts used for the nutrient solution are stated in Supplementary data, Tab. 2).

7.2.5 Sample analysis

The duration of the phytoremediation experiment is 30 days, samples of 5 ml volume are taken on days 0, 1, 2, 4, 7, 14 and 30. The sampling strategy is planned with respect to plants state, as explained in section 3.2. In this work only samples from the liquid solution are considered, the plants themselves are not exploited to any additional analysis (i.e. tissues examination). The samples are filtered through 0.45 µm syringe (Carl Roth GmbH). To monitor the uptake of inorganic salts and MPs by plants, concentration of dissolved oxygen and other parameters, pH, electrical conductivity, oxidation-reduction potential are measured on sampling daily base. Between every measurement, the electrodes are washed carefully with Milli-Q water to avoid any cross-contamination. The concentrations of nitrate, phosphate and COD (Hach Lange test cuvette set) are measured on site of the laboratory on each day of sampling as well. After filtration, the samples are frozen to -20 °C and then analyzed externally in Luxembourg Institute of Science and Technology (LIST).

The samples are filtered and diluted with water/methanol 90/10, as pre-conditioning is not needed and then immediately analyzed by Liquid Chromatography (1260 Series, Agilent, Santa Clara USA) coupled to triple-quadrupole Mass Spectrometry (QTRAP 4500, AB Sciex, Framingham USA).

The chromatographic separation is carried out on Zorbax Eclipse Plus C18 column, 150 × 2.1 mm ID, 3.5 µm particle size (Agilent). The flow rate of the mobile phase is constant at 0.25 mL/min and the oven temperature remains at 40 °C. The mobile phases used are based on ultrapure water and LC-MS grade acetonitrile buffered with LC-MS grade formic acid (0.1%, positive mode) or ammonium acetate (2.5 mM, negative mode). Considering the target compounds, the mass spectrometer is operated in positive or negative electrospray ionization, in Multiple Reaction Monitoring (MRM) mode. Two MRM transitions are used for each compound of interest, for quantification and confirmation, respectively [316]. Quantitative results are provided thanks to internal calibrations. Quantification limits are provided in Supporting Documents.

7.3 Results and discussion

7.3.1 General parameters

In the last days of experiment, a high level of COD has been detected (in orders of g/l). High concentration of COD can probably be related to the presence of methanol as a solvent for MPs in the nutrient solution. Additionally, due to inner metabolic plant processes, young plant leaves may produce much higher methanol emissions, than leaves, which already reached maturity [317]. Furthermore, mechanical damage, which could happen during the removal of necromass and during the replacement of plants from soil into aqueous solution and also presence of pathogens [318] can lead into dramatic increase of methanol concentration [319]. Besides the facts mentioned above, the stress, to which plants are exposed and slightly higher temperatures (max. 25 °C) could lead to increased concentration of biofilm, which could cause in the end effect also slow lysis of plants (confirmed also by decreasing values of oxidation-reduction potential and dissolved oxygen, see supplementary data), accompanied by release of methanol, which is abundantly present in plant cells.

pH level for all controls have been stable for the whole period of the experiment (7.0 ± 0.2). The conductivity level remains steady as well, in range between 1100 ± 100 μS/cm. Concentrations of phosphate in the liquid solution remained in all controls 98.0 ± 2.2 mg/l. For further information, see supplementary data.

Concentration of nitrate in the liquid solution is visibly decreasing from 7th day of operation (Fig. 7.1)

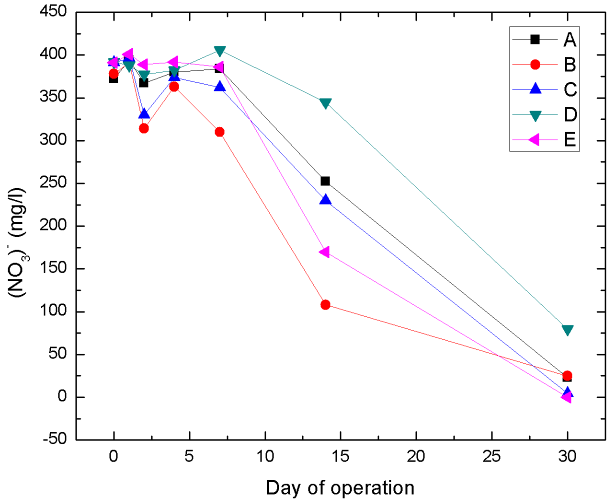

Fig. 7.1. Decreasing concentration of (NO_3)- in solutions of all controls (A – Phragmites fresh, B – Iris fresh, C – Lythrum fresh, D – Phragmites adapted, E – Iris adapted).

Nitrate belongs to the most essential nutrients needed for plant growth and as such it is also easily adsorbed by the plants [320].

As mentioned in chapter 7.2..4, the conditions for hydroponic culture have been optimized. Under these optimized conditions, it is observed that controls A, B and C show growth of healthy phytomass, which confirms the right choice of parameters (Fig. 7.2).

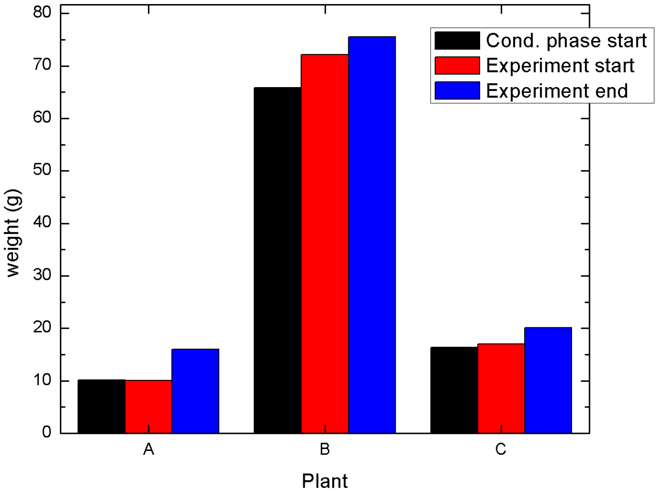

Fig. 7.2. Growth development of controls A, B and C at the beginning of conditioning phase, at the end of the conditioning phase = at the beginning of the experiment with MPs and at the end of the experiment with MPs.

7.3.2 Micropollutants

Due to the fact, that a broad variety of MPs for phytoremediation by different plant species is studied, the exact determination of the removal mechanisms (e.g. volatilization, accumulation) is not the purpose of this work. In general, it is known that MPs are accumulated in plants tissues [321], where they can be transported with the translocation stream (e.g. antibiotics [184], in this case probably ciprofloxacin, clarithromycin, erythromycin), eventually the compounds accumulate also in stems or roots. Low-molecular-weight compounds can be transported through the plant and released from the leaves by phytovolatilization [184] (in this case possible at e.g. AMPA or benzotriazole). The uptake is dependent on the type of the plants, concentration and character of the MPs. Incorporated to CWs, mentioned removal mechanisms are influenced by the presence of biofilm, animal and microbial communities in the wetland [280,322,323].

After conditioning phase, at the beginning of the phytoremediation experiment is the uptake of MPs expected to be most rapid, because the plants are already used to hydroponic conditions and they are recovered from the significant change of environment (from soil into liquid solution). This is also confirmed in literature [234,307]. The phytotoxic effect needs to be considered as well, because some of the MPs, e.g. antibiotics [184] and herbicides have toxic effect on the plants and this can cause their deteriorative uptake abilities. We suppose, that the longer the plants are exposed to the MPs, the bigger is the phytotoxic effect (accompanied by increasing COD values and decreasing values of oxidation-reduction potential [324] and dissolved oxygen [325]) and their removal efficiency weakens with time, as shown on case of ciprofloxacin in Fig. 7c). This is a reason why the sampling for MPs content is performed more often at the beginning of the experiment [307,326].

Removal rate (r.r.) is calculated from following equation: $r.r.(\%) = \frac{c_0 - c}{c_0} * 100\ \%$, where c_o is initial concentration of the MPs and c is the concentration in given day of the experiment. The most surprising fact is, that PFOA and PFOS, which are compounds known to be hardly eliminated from wastewater [327,328] are removed in this experiment with efficiency 15 – 27 % in case of PFOA (Fig. 7.3a) and 21 – 30 % in case of PFOS by both controls of *Phragmites*. On the other hand, this phenomenon is also shown in literature, where plants exposed to increased concentrations of perfluoroalkyl acids show increased removal of these substances, esp. PFOA [288]. *Iris pseudacorus*, shows also remarkable removal efficiency for MPs from group of beta-blockers, especially propranolol (50 %, Fig. 7.3b), followed by atenolol (35 %) and metoprolol (31 %). Removal efficiencies of beta blockers confirm usefulness of this method as well, because they are comparable or higher, than observed during removal of these compounds in WWTPs e.g. in Canada [329]. Erythromycin and clarithromycin (macrolide antibiotics), as well as ciprofloxacin (broad fluoroquinolone antibiotic), are removed with high efficiency especially in case of *Lythrum* and *Iris* (90 % for ciprofloxacin, 35 % for erythromycin and 32 % for clarithromycin). However, the quantitative determination of macrolides may be affected from experimental uncertainties because possible synthesis intermediates are not measured. Phytoremediation seems to be also relevant especially for ciprofloxacin, which remains in wastewater effluent even after ongoing treatment [330] and so could be removed by the polishing step applying the plants mentioned in this work. The removal efficiency of all plants is demonstrated in the case of removal of ciprofloxacin (Fig. 7.3c). Higher passive adsorption of ciprofloxacin by *Phragmites australis* is observed also by other investigations, when the plant is exposed to higher amounts (mg/l) of this antibiotic [184,331]. Compounds, which have lower log K_{OW} are more hydrophilic [332] (Tab. 7.1) and therefore are better soluble in water. These compounds (e.g. atenolol, ciprofloxacin, cyclophosphamide) seem to be better removed by the plants, which is also confirmed in literature [333,334].

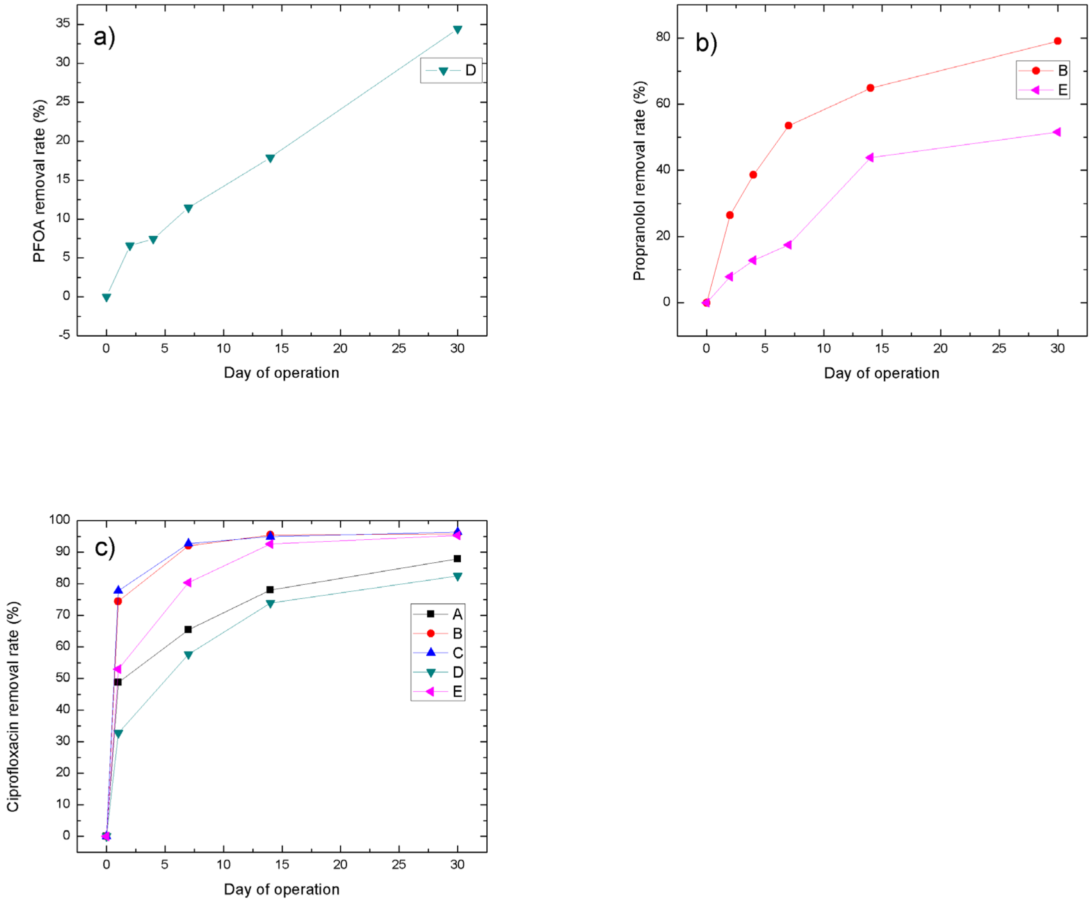

Fig. 7.3. R. r. of specific MPs: Removal of PFOA by control D (a), removal of propranolol by both types of *Iris*, B – fresh, E – adapted (b) and removal of ciprofloxacin by all used plants (c).

The initial and final concentrations of studied MPs of controls, A – E are stated in Supplementary data. In general, the best removal of MPs is visible in case of the control C – *Lythrum* fresh, where the highest r. r. of all MPs exceeds 20 %, except of the case of Ketoprofen and AMPA (Fig. 7.4). However, in case of AMPA the information can be misleading, as AMPA is a degradation product of glyphosate and it has tendency to retransform back to glyphosate [238,239]. The ability of *Lythrum salicaria* to remove pollutants from aqueous solutions has been confirmed in experiments of removal of e.g. heavy metals [289] and herbicides [335].

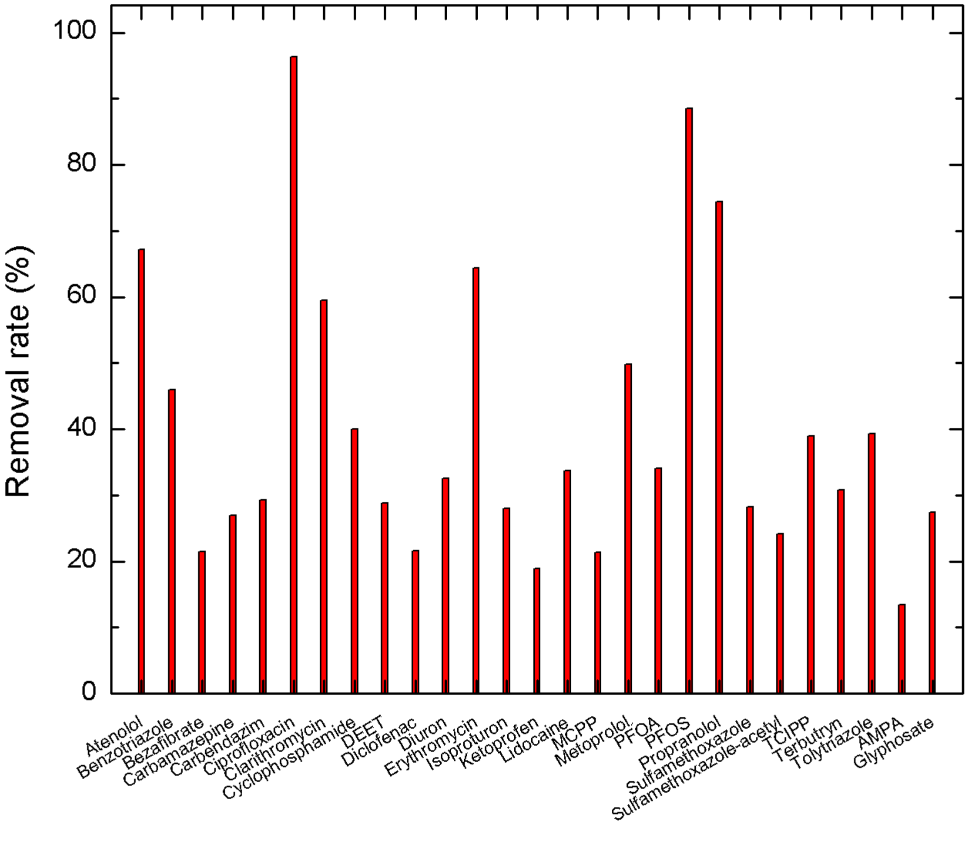

Fig. 7.4. Removal of 27 compounds by *Lythrum salicaria*.

On the contrary, both cases of Phragmites, fresh (A) and the one adapted to wastewater (D), show the lowest r.r. of all the compounds.

In cases of *Phragmites*, the average removal ability is higher at the control D, which was used previously for treatment of wastewater effluent in WWTP Reisdorf (esp. in cases of atenolol, clarithromycin and erythromycin, Fig 7.5a). Surprisingly, both of these plants show good removal abilities in cases of fluorosurfactants (PFOA 15 – 27 %, PFOS 21 – 30 %). In cases of *Iris*, the r.r. speak in advantage of the control B, the fresh *Iris* (esp. in cases of beta-blockers, Fig. 7.5b).

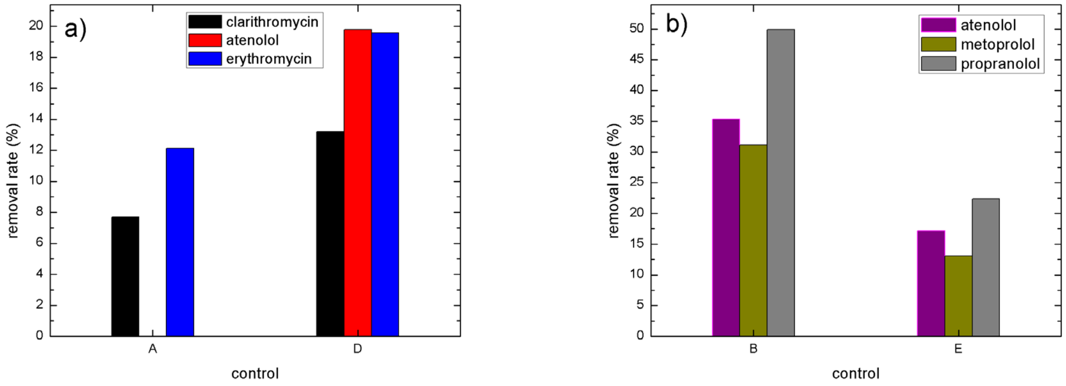

Fig. 7.5: Comparison of average removal efficiency of controls A vs. D (a) and B vs. E (b).

Because *Phragmites* does not have very dense stems and it has relatively shallow root system [336], it grows in so-called reed beds [111]. For our experiments, just single stems with roots are used. That is probably why the removal ability of the control D, *Phragmites* adapted, is higher, than the ability of the control A, *Phragmites* fresh, because *Phragmites* adapted had longer vegetation period, stronger stems and leaves, from the physiological point of view is probably able to uptake bigger amount of MPs. On the other hand, *Iris* is well known for its strong, bright leaves, growing directly in a fan from the soil [337]. Both controls, the fresh one and the adapted one, are in a similar physiological status. However, since *Iris* adapted has been used for treatment of wastewater effluent for 16 months, it is possible that its capabilities to accumulate MPs are not so strong anymore. That might be a reason, why fresh *Iris* shows better r.r. of micropollutants compared to *Iris* adapted.

7.4 Conclusions

- One of our goals was to establish optimal conditions for the plants in hydroponics, which we determine as: additional support of the plant growth with LED lamp, proper choice of the nutrients, prolonged acclimatization period and ensured oxygen supply. We found out, that stable level of pH and conductivity are essential for steady conditions of the hydroponic culture as well, which are then preferable for phytoremediation. These findings are helpful for further optimization of hydroponics, which has a broad usage in many domains of the environment.
- Phytoremediation as an independent process seems to be relevant polishing technology for wastewater effluents especially in cases of fluorosurfactants (r.r. up to 30 %), beta-blockers (r.r. up to 50 %) and antibiotics (r.r. up to 90 %).
- In CWs, phytoremediation contributes significantly to overall removal efficiency thanks to the ability of plants to take up specific MPs from wastewater.
- In this experiment, it was found out, that *Lythrum salicaria* has a potential for further usage in phytoremediation of emergent pollutants from aqueous solutions, because it showed removal efficiency more than 20 % for 25 out of 27 compounds.
- Based on our experience, we recommend to use *Phragmites australis* for phytoremediation experiments in a more mature state or in higher stem density per m^2 (e.g. reed beds), due to its physiological arrangement. On the other hand, we suggest to apply *Iris* in an early stage of its vegetation state.

These conclusions summarize the main outputs of the work, they state clearly the results and give a recommendation for further investigation in phytoremediation processes and optimization of hydroponic conditions.

Acknowledgement

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8 Publication IV - Bioremediation of 27 Micropollutants by Symbiotic Microorganisms of Wetland Macrophytes

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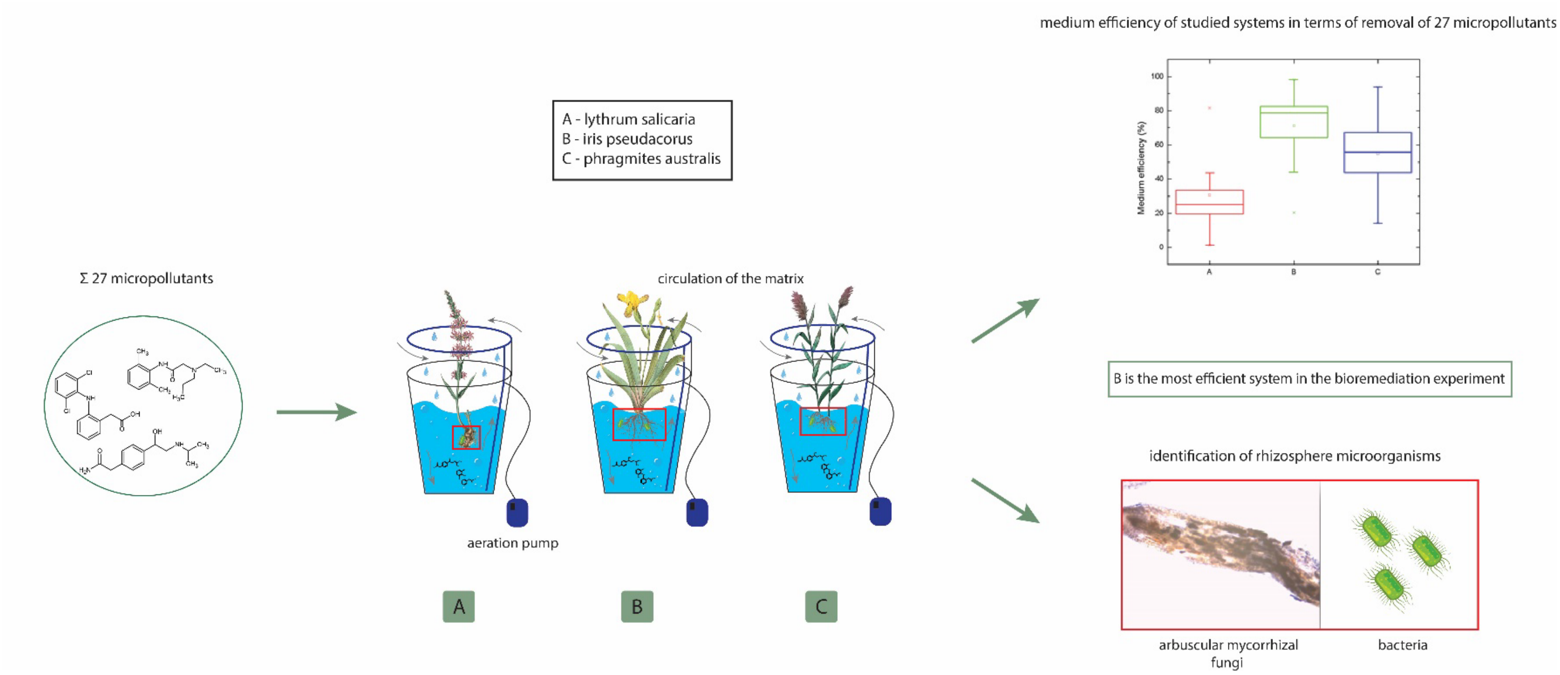
Graphical abstract



Abstract

Background: Micropollutants in bodies of water represent many challenges. We addressed these challenges by the application of constructed wetlands, which represent advanced treatment technology for the removal of micropollutants from water. However, which mechanisms specifically contribute to the removal efficiency often remains unclear. Methods: Here, we focus on the removal of 27 micropollutants by bioremediation. For this, macrophytes *Phragmites australis*, *Iris pseudacorus* and *Lythrum salicaria* were taken from established wetlands, and a special experimental set-up was designed. In order to better understand the impact of the rhizosphere microbiome, we determined the microbial composition using 16S rRNA gene sequencing and investigated the role of identified genera in the micropollutant removal of micropollutants. Moreover, we studied the colonization of macrophyte roots by arbuscular mycorrhizal fungi, which are known for their symbiotic relationship with plants. This symbiosis could result in increased removal of present micropollutants. Results: We found *Iris pseudacorus* to be the most successful bioremediative system, as it removed 22 compounds, including persistent ones, with more than 80% efficiency. The most abundant genera that contributed to the removal of micropollutants were *Pseudomonas, Flavobacterium, Variovorax, Methylotenera, Reyranella, Amaricoccus* and *Hydrogenophaga*. *Iris pseudacorus* exhibited the highest colonization rate (56%). Conclusions: Our experiments demonstrate the positive impact of rhizosphere microorganisms on the removal of micropollutants.

*Keywords***:** arbuscular mycorrhizal fungi; bioremediation; constructed wetlands; removal of micropollutants; rhizosphere microbiome

8.1 Introduction

With rising globalization, industrialization and the world population in general, the use of synthetic chemical compounds continues to grow. Many of these compounds are micropollutants (MPs); released broadly, their removal from bodies of water is an enormous challenge [1]. This is due to the fact that current conventional wastewater treatment plants (WWTPs) are not designed for MPs' removal and, therefore, a high percentage of MPs remains in the WWTPs' effluents, which are then discharged into bodies of water [2]. MPs cause negative effects on aquatic fauna and flora [6], induce mutagenicity [11], contribute to antibiotic resistance [13] and, consequently, have negative impacts on human health [10]. Because of this, the European Commission (EC) decided to require mandatory monitoring of some MPs (i.e., antibiotics, such as azithromycin, clarithromycin and erythromycin) by all European Union (EU) member states, with the ultimate goal of preserving the ecological and chemical status of the surface bodies by 2027 [15–17].

This also contributed to the active research and introduction of advanced treatment technologies in the last twenty years. Advanced oxidation processes, UV photolysis, ozonation and membrane applications are widely used; however, these technologies have high financial requirements and are challenging to implement (e.g., due to the space requirements) [338–340]. According to the principles of the 2030 agenda for sustainable development, with rising urbanization, there is a growing demand for the presence of nature in urban islands promoting resilience [341,342].

Constructed wetlands (CWs) are a possible solution to these challenges and offer distinct environmental advantages. CWs act as attractive biodiverse enhancements in many urban areas [343,344] and have recently been reported as useful for MPs' removal [345–347]. We investigated CWs in our recent project, EmiSûre (Interreg, N 013-2-03-049), where, for most of the 27 investigated compounds, the overall removal efficiencies of vertical subsurface flow CWs, as a post-treatment step, exceeded 90% [348]. In order to learn from this experiment, a further aim has been developed to quantify the MPs' removal mechanisms in the studied wetlands and understand the individual contributions. These mechanisms can be divided as follows: 1. phytoremediation by wetland macrophytes, 2. adsorption on the soil matrix, and 3. bioremediation by microorganisms. Phytoremediation is important in horizontal configurations but is considered negligible for subsurface flow CWs, especially in vertical configurations where the surface exposed to sunlight is limited [227]. Phytoremediation and adsorption have already been targeted in our previous studies [228]. With the knowledge gained from phytoremediation, we could compare and improve our current set-up and assess the efficiency of pure plants for removing MPs. During the phytoremediation experiments, the roots were immersed into a biocidal solution to exclude the presence of microorganisms in the root zone. With this information, it is possible to develop an innovative experimental set-up for the establishment of bioremediation and appraise the additional contribution of the rhizosphere organisms, which we suppose to be significant, as the rhizosphere is known to be the most reactive zone of a wetland [349]. Bioremediation has gained increased attention in recent years, as it is a non-invasive and natural way of eliminating MPs. There are recent studies on the application of the bioremediation of MPs present in irrigation water. The implementation of biochar in bioremediation, which is an effective substrate for MPs' removal, was also in our previous applications [348,350,351]. The aqueous environment can have bioremediation effects influenced by various factors, such as the presence of commonly occurring MPs (e.g., bisphenol A), which enhance bacterial growth [352] and the beneficial relationships between plants and microbes [353,354].

In order to complete the understanding of the mechanisms' removal, the characterization of the present microorganisms, namely bacteria and fungi, was performed. In order to characterize the bacterial community and to determine the role of selected genera in the studied systems, 16S rRNA gene amplicon sequencing [355] was applied to samples of roots and soil. Wetland bacteria are known for the degradation of an expansive variety of nutrients and inorganic and organic compounds. Most bacteria degrade broad groups of compounds, e.g., *Reyranella* or *Rhodobacter* decompose organic matter and, therefore, play major roles in the removal of petroleum pollutants [356]. For instance, *Hydrogenophaga* is a genus of known general benzene degraders [357]. Other bacterial genera that target specific compounds are *Massilia,* which decompose tris (1-chloro-2-propyl) phosphate (TCIPP) [358], and *Sphingobium,* which include known diclofenac degraders [359].

Besides bacteria, we also studied arbuscular mycorrhizal fungi (AMF), which are commonly present in wetlands [111]. The symbiotic relationship of these soil-borne fungi with plants belongs to the most important ones on Earth [360], as they are found in over 80% of all plant species [361]. AMF can also enhance phytoremediation by creating an underground network from mycelium, which acts as a bridge between plant roots, soil and microorganisms in the rhizosphere. The hyphae of AMF can significantly increase the access area of the plant to nutrients and contaminants. Therefore, AMF contribute to bioremediation because they considerably increase the active root area for the uptake of pollutants [362]. AMF provide host plants with nutrients, such as phosphorus and nitrogen; host plants transfer 4 to 20% of photosynthetically fixed carbon to fungi. The presence of AMF spores generally decreases with soil depth, and the spores are normally absent below the root zone [363]. AMF colonization can be influenced by environmental parameters, such as, (1) flooding conditions [364], (2) temperature (the colonization rate increases with the growth of the temperature from 10 to 30 °C) [365], (3) level of oxygen (the decrease of colonization is between 21 and 3% of oxygen and concentration of oxygen below 3% cases abrupt decrease of the colonization) [366], and (4) pH (the maximum spore germination occurs between pH 6 and 8) [365]. For the contribution of the AMF to the phyto- and bioremediative activity of wetlands, a colonization of the plant roots by the AMF has been examined in this work.

Overall, the main aim of this work is to understand the bioremediation process and its contribution in a CW environment to the removal of MPs. The hypotheses are (1) the rhizosphere is the most active area in which the removal of MPs occur, and (2) fungi and bacteria in the rhizosphere are crucial in the removal process. Thus, in order to better understand this, we designed a new experimental set-up. Consequently, it will be possible to: (1) evaluate the bioremediative potential of the wetland macrophytes with organisms present in the rhizosphere for the removal of MPs; (2) characterize the available bacterial microbiome, aiming to understand their function better; and (3) relate the MPs' removal of bacterial genera with the presence of AMF determined by the colonization of plant roots. Ultimately, it will be possible to offer advice on how to enhance the potential of the rhizosphere in the removal of MPs via CWs.

8.2 Materials and Methods

8.2.1 Design of a Bioremediation Experiment

Three common wetland macrophytes (*Lythrum salicaria* (A), *Iris pseudacorus* (B) and *Phragmites australis* (C)) previously purchased at re-natur GmbH (Ruhwinkel, Germany) were taken from an established pilot-scale CW. Our usage of the plants did not disregard any of the legal conservation guidelines. In the CW, bentonite sand and a 15% activated biochar admixture acted as a substrate. The wetland was tested in the WWTP Echternach (20,000 PE equivalent capacity, Luxembourg) as a post-treatment step. When removing the plants, the excess soil was removed, leaving just the soil present in theroot area (rhizosphere). This was due to the preservation of the rhizosphere microbiome, which should contribute to the removal of MPs. The samples from the rhizosphere soil, with the roots of the macrophytes, were sampled with sterilized tools and immediately put in a liquid nitrogen dry shipper (Voyageur–Dry

Shippers (2–Plus) AIR LIQUIDE Medical GmbH, Düsseldorf, Germany). The "systems" (plants with present rhizosphere microbiome) were placed into special hydroponic pots (Growrilla Hydroponics, Ciriè, Italy) with tap water for one day for conditioning. The pots contained an aeration unit, which ensured the sufficient oxygenation of the plants' roots and constant recirculation of the liquid medium in the pot (Figure 8.1).

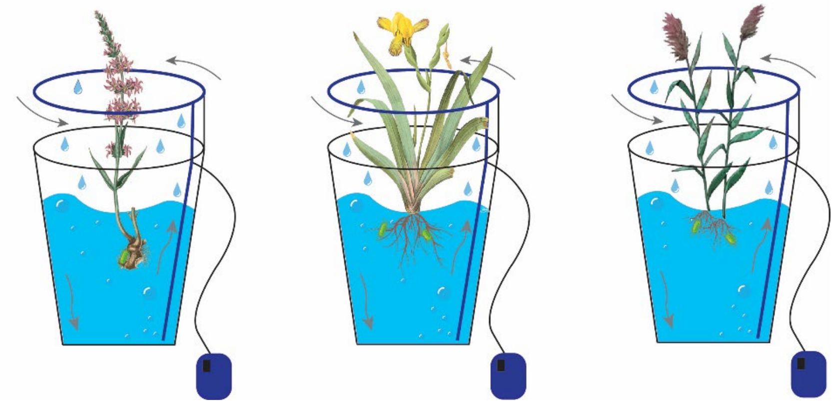


Figure 8.1. Scheme of the bioremediation experiment.

After one day, the tap water was withdrawn from the pots. Then, a mixture of 27 MPs in concentrations of 1–5 µg/L (Techlab, purity >99.99%) was added to the pots. This concentration is typical for small-to-medium sized WWTP effluents and hydroponic nutrients in water (15 L) (Flora Series, General Hydroponics—the detailed composition of the nutrient solutions is available in Supplementary Materials). The list of MPs is shown in Table 8.1.

Table 8.1. MPs studied in this work.

Application	Compound	CAS Number	Therapeutic Group/Use
Pharmaceuticals and metabolites	Atenolol	29122-68-7	Beta Blocker
	Bezafibrate	41859-67-0	Lipid regulator
	Carbamazepine	298-46-4	Psychiatric drug
	Clarithromycin	81103-11-9	Antibiotic
	Ciprofloxacin	85721-33-1	Antibiotic
	Cyclophosphamide	50-18-0	Cytostatic
	Diclofenac	15307-86-5	Analgesic/anti-inflammatories
	Erythromycin A	114-07-8	Antibiotic
	Ketoprofen	22071-15-4	Analgesic/anti-inflammatories
	Lidocaine	137-58-6	Anaesthetic
	Metoprolol	51384-51-1	Beta Blocker
	Propranolol	525-66-6	Beta Blocker
	N4-acetylsulfamethoxazole	21312-10-7	Metabolite of Sulfamethoxazole
	Sulfamethoxazole	723-46-6	Antibiotic
Pesticides/Herbicides	Carbendazim	10605-21-7	Fungicide
	DEET	134-62-3	Insect repellent
	Diuron	330-54-1	Herbicide
	Isoproturon	34123-59-6	Herbicide
	Terbutryn	886-50-0	Herbicide
	Mecoprop (MCPP)	7085-19-0	Herbicide
	Tolyltriazole	29385-43-1	Fertilizer
	Glyphosate	1071-83-6	Herbicide
	Aminomethylphosphonic acid (AMPA)	1066-51-9	Degradation product
Fluorosurfactants	Perfluorooctanesulfonic acid (PFOS)	1763-23-1	Surfactant
	Perfluorooctanoic acid (PFOA)	335-67-1	Surfactant
Corrosion inhibitor	Benzotriazole	95-14-7	Corrosion inhibitor/Antiviral
Flame retardant	Tris(2-chloroisopropyl)phosphate (TCPP)	13674-84-5	Flame retardant

The pots were lighted with a LED lamp for hydroponic plants, which included 96 LED chips (32 yellow beads, 32 blue beads, and 32 red beads), and a wavelength of 380–800 nm at 36 watts (Lovebay International Limited, Bristol, England), for 12 h per day. The duration of the experiment was 30 days, with sampling on days 0, 1, 2, 5, 7, 14, and 30 (analogous to our phytoremediation experiment [228]). The volume of each sample was 100 mL. The samples were, subsequently, filtered through a 0.45 µm syringe (Carl Roth, GmbH, Karlsruhe, Germany), and the content of the macronutrients and values of the general parameters were analyzed on-site (COD, TN, NO_3^-, NH_4^+, PO_4-P (Hach Lange cuvette text box), electrical conductivity, oxidation-reduction potential (ORP), dissolved oxygen (DO), and pH (multi-portable parameter meters by Xylem Analytics Germany Sales GmbH & Co. KG, Wilhelm in Oberbayern, Germany)). The concentrations of the MPs were measured at the Luxembourg Institute of Science and Technology (LIST) [228].

8.2.2 Microorganisms

In order to determine the bacterial composition, samples of the plants' roots and the rhizosphere were taken and immediately placed in a liquid nitrogen dry shipper (Voyageur–Dry Shippers (2–Plus) AIR LIQUIDE Medical GmbH, Düsseldorf, Germany). Next, root and soil samples were prepared for DNA extraction at the Luxembourg Centre for Systems Biomedicine (LCSB). First, the samples were milled and homogenized under cryogenic conditions at −196 °C (6875D Freezer/Mill® Dual-Chamber Cryogenic Grinder SPEXSamplePrep). After homogenization, the DNA was extracted according to standardized procedures (DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) and PowerSoil DNA Isolation Kit (MOBIO Laboratories, Inc., Berlin, Germany). The extracted DNA was concentrated and purified. The DNA's quality and quantity were assessed using a nanophotometer (Nanodrop) and fluorometer (Qubit dsDNA HS Assay Kits, Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the sample preparation, sequencing (Oxford Nanopore Technologies MinION sequencer) and data analysis, including the taxonomic classification, were carried out by the LCSB Sequencing Platform (RRID SCR_021931) at the University of Luxembourg using the protocols provided by the manufacturer.

The roots were examined for the presence of AMF. First, the roots (more than 100 pieces per plant species) were cleaned under a water stream and cut into 1 cm pieces. Next, the roots were cleaned in a 10% KOH solution [366] and stained in an ink and vinegar solution [367]. Then, the colonization of the macrophytes' roots by AMF, before and after the targeted experiment, was evaluated with the help of the grid-line intersect method and microscopical observation (LMS Leica DM1000, Düsseldorf, Germany, zoom 10x).

8.3 Results

8.3.1 General Parameters and Macronutrients

We observed a rapid increase in the removal efficiency of the COD (chemical oxygen demand) within the first days (Figure 8.2). The efficiency of system A dropped slightly towards the end of the experiment on day 30. The efficiency of system B continued to increase, reaching 84% on day 30 slowly. It was also the highest removal efficiency that we observed across the three plant species studied herein.

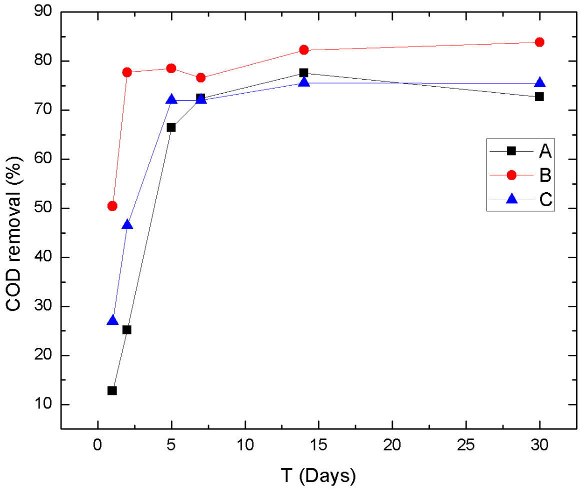


Figure 8.2. COD removal via bioremediation. A = *Lythrum*, B = *Iris*, and C = *Phragmites*.

The values of TN (NO_3^- + NH_4^+) (160–220 mg/L) and PO_4^--P (38–41 mg/L), which were monitored during the entire experiment, were in line with the recommended values for these nutrients (100–250 mg/L for TN and 30–50 mg/L for PO_4^--P [368]. The plants were taken from the CW after the winter season; therefore, they had a comparably low green biomass. During the experiment in semi-hydroponic conditions, the plants underwent a significant increase in healthy biomass (80–100 cm of new stems for each plant and 10–30 cm of roots for each plant). These findings suggest well-established hydroponic surroundings and, therefore, positive prerequisites for an optimal symbiotic relationship between the rhizosphere organisms and the plant roots, which results in a favorable environment for the removal of MPs from the liquid solution.

During the experiment, a constant decrease in the concentration of NH_4+ ions was observed (from 25 to 3 mg/L). The concentrations of NO_3- ions remained constant during our experiments (60–100 mg/L). Moreover, the values of DO remained stable (6.6–7.9 mg/l corresponding to 71–85% oxygen saturation), considering that the concentration of the DO at a saturation point of 20 °C is 9.1 mg/L [369]. These facts suggest an ongoing nitrification process, where NH_4+ is oxidized to NO_3-. This could help the removal of MPs, as many of the nitrifying bacteria are known for their ability to degrade organic compounds [370]. However, it is not possible to confirm this hypothesis surely, as it is not clear which amount of NO_3- is being up taken by the plants and which amount is oxidized from NH_4+. The measured values of pH and ORP during this experiment are available in the Supplementary Materials.

8.3.2 Removal of Micropollutants

The ability of the studied systems to remove MPs from the liquid medium is delineated as follows:

The most efficient system for the removal of MPs is macrophyte B, Iris, which removed 22 out of 27 compounds with more than 80% efficiency. The successfully removed compounds were atenolol, benzotriazole, bezafibrate, carbendazim, ciprofloxacin, clarithromycin, cyclophosphamide, DEET, diclofenac, diuron, erythromycin, glyphosate, isoproturon, ketoprofen, MCPP, metoprolol, propranolol, sulfamethoxazole, and its acetyl degradation product, TCIPP, tebutryn, and tolyltriazole. Table 8.2 shows a comparison of the bioremediation removal of the compounds in the current experiments and the bioremediation experiments described in the literature.

Table 8.2. Removal of 22 compounds in the current study compared to achieved removals in previous studies.

Compound	Achieved Removal in Current Study (%)	Achieved Removal in Previous Studies	Reference
atenolol	98.8	80%	[371]
benzotriazole	93	complete removal, however conditioned by low concentration of the compound	[372]
bezafibrate	99.9	contribution of the biofilm to removal of 25%	[176]
carbendazim	99.3	41.8%	[373]
ciprofloxacin	99.5	contribution of the biofilm to removal of 22%	[176]
clarithromycin	99.4	75.8–98.6%	[374]
cyclophosphamide	91.8	>20%	[375]
DEET	99.6	no significant removal	[376]
diclofenac	99.7	97 ± 4%	[377]
diuron	99.7	83%	[378]
erythromycin	98.3	75.8–98.6%	[374]
glyphosate	99.2	82.6%	[379]
isoproturon	99.6	complete removal	[199]
ketoprofen	99.9	complete removal	[377]
MCPP	99.5	99%	[380]
metoprolol	91	60%	[371]
propranolol	98.9	60%	[371]
sufamethoxazole	90.5	75.8–98.6%	[374]
N-acetyl-sulfamethoxazole	99.5	no information founded	
TCIPP	89.9	60%	[358]
terbutryn	99.6	complete removal	[381]
tolyltriazole	95.7	complete removal	[372]

From Table 8.2, it is clear that the previously mentioned experimental set-up could be a solution for the removal of compounds such as beta-blockers, carbendazim, cyclophosphamide, DEET and TCIPP, which were not well-removed by bioremediation before.

Among the plants, Iris did not prove to perform the best during our phytoremediation experiments carried out in the past, probably because it was not very well-developed. A comparison between the removal efficiency of Iris during the phyto- and bioremediation experiments is shown in Figure 8.3.

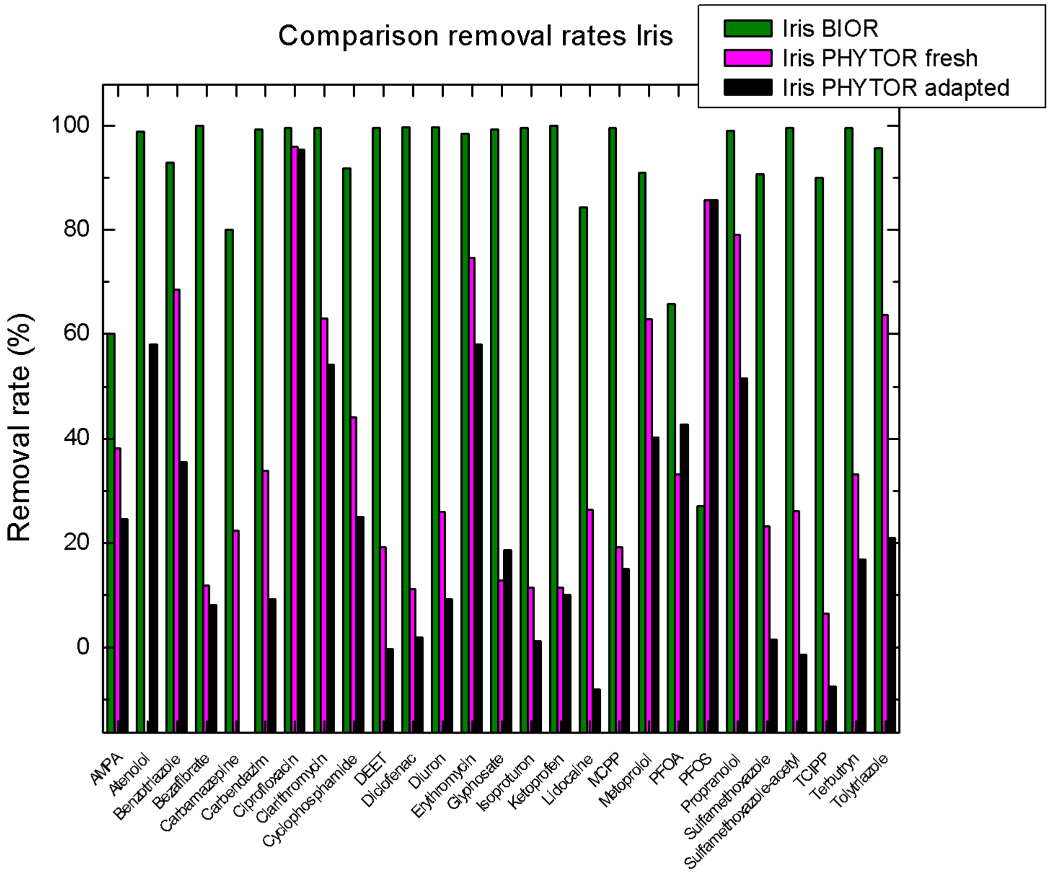


Figure 8.3. Comparison of the removal efficiency of Iris during bioremediation and fresh and adapted Iris during phytoremediation.

The removal rate (*R. r.*) was calculated using the following equation: $R.r.(\%) = \frac{c_0 - c}{c_0} * 100\ \%$, where co is the initial concentration of the MPs and c is the concentration on any given day of the experiment. With microorganisms present in the rhizosphere, the remediation system resulted in a higher MP removal than for plants without rhizosphere present. Focusing on the performance of Iris with the presence of the rhizosphere, it is apparent that some compounds are removed with medium-to-poor efficiency (<80%):

- AMPA, which was, notably, not removed from our CWs' installations (Venditti et al., 2022), is a degradation product of glyphosate that tends to retransform back to its maternal compound [238,239].

- Carbamazepine, which is a poorly biodegradable compound, and its metabolites can build back to the parent compound. Therefore, removal is not assumed in conventional WWTPs [375], while in the presented experiments, this compound was removed up to 80%.
- Fluorosurfactants, in this case, PFOA and PFOS, are generally persistent compounds that tend to accumulate in the surrounding media [382] and, in the present study, were removed up to 66% (PFOA) and 27% (PFOS).

We can demonstrate the usefulness of this removal process by providing two insights: First, the adapted method with a continuous oxygen supply (due to aeration) reduces stress in the rhizospheric system (anoxic conditions), and the permanent mixing of the aqueous solution guarantees representative sampling. Therefore, the configuration and design of the experiment represent the bioremediation process and show the importance of the rhizospheric system. Nevertheless, sufficient oxygen levels seem to be essential for the MPs' removal in the rhizospheric system under real conditions. Additional forced aeration and recirculation of wastewater have previously demonstrated an increase in the aerobic capacity of the system and, thus, could be advantageous for the removal of MPs by CWs [383–385]. Second, poorly biodegradable or persistent MPs, such as metoprolol [191] and lidocaine [386], were removed by 91% and 84%, respectively. TCIPP, which passes through conventional wastewater treatment [387] and persists in treatments by advanced technologies, was removed by our approach up to 90%. The concentration profiles of all the compounds in each system, together with the quantification limits, are available in the Supplementary Materials.

In our previous phytoremediation experiments, *Lythrum* was the most efficient macrophyte. In the present bioremediation experiments, *Lythrum* exhibited the lowest MP removal efficiencies. This is probably due to its weakened physiological status after the winter period. A comparison of the medium efficiency of the MPs' removal by the three macrophyte bioremediative systems is shown in Figure 8.4.

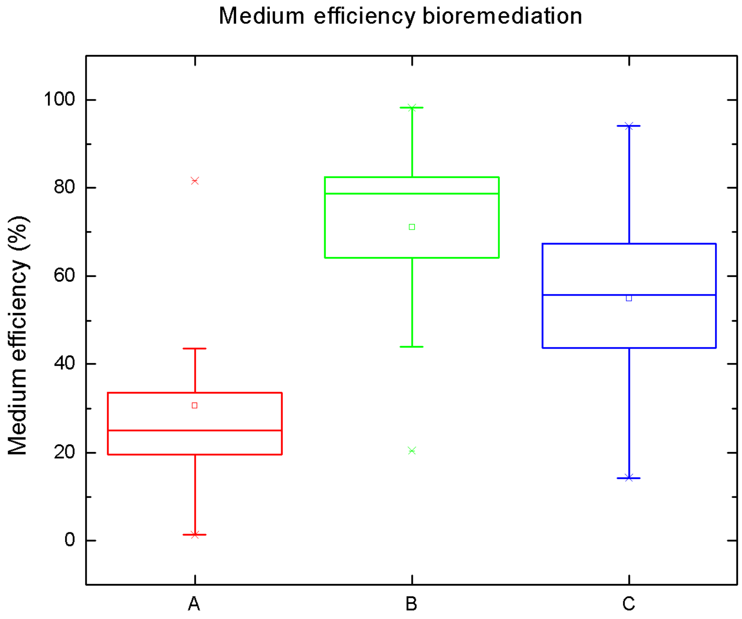


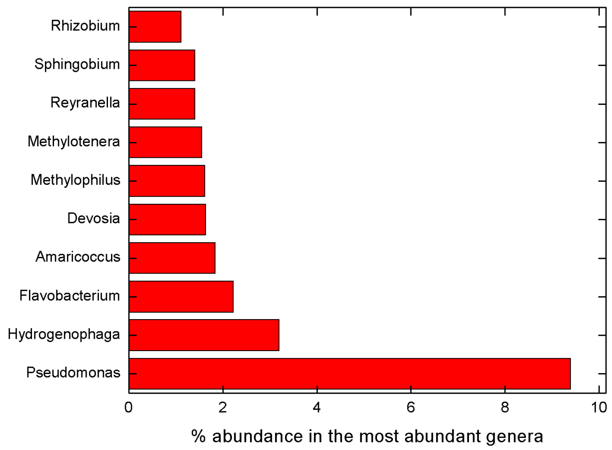
Figure 8.4. Medium efficiency of MPs’ removal of the studied macrophyte systems, (A = *Lythrum*, B = *Iris*, and C = *Phragmites*).

Overall, our results suggest that the role of the rhizosphere in a CW environment could be substantially enhanced if additional aeration conditions and sufficient nutrients are provided.

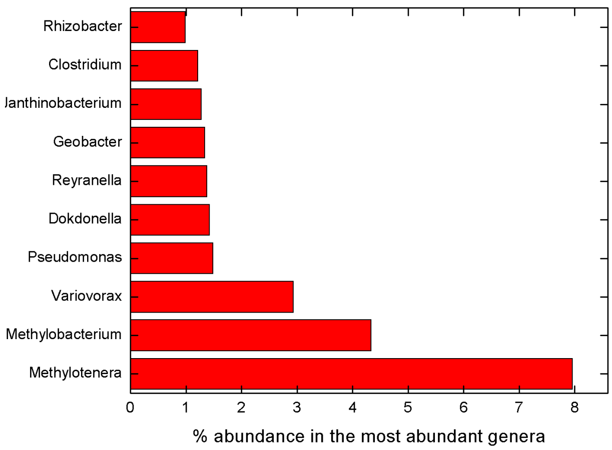
8.3.3 Microbial Composition

In order to better understand the rhizosphere microbiome in CWs, we studied the bacterial complement by sequencing the 16S rRNA gene of this microbiome. For simplification, we focused on the most abundant 25 genera (a list of these genera is available in the Supplementary Materials), which represent the majority (>68%) of the overall population. Of these, we focused on bacteria with known potential for MPs’ removal. The total abundance of these genera varied from 25–40%. The following figures (Figure 8.5) show the detailed abundances of genera known for the removal of organic MPs in the studied samples.

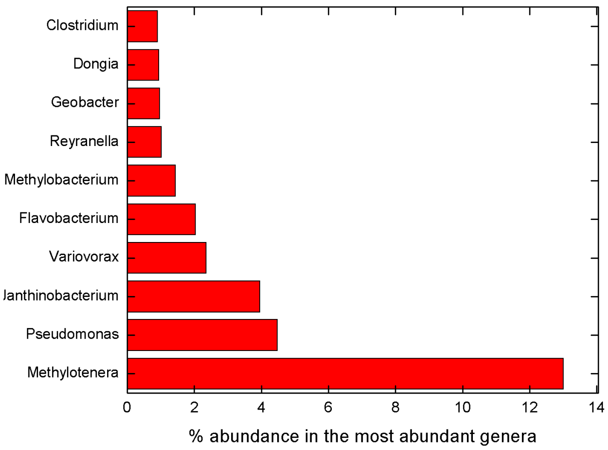
BPB



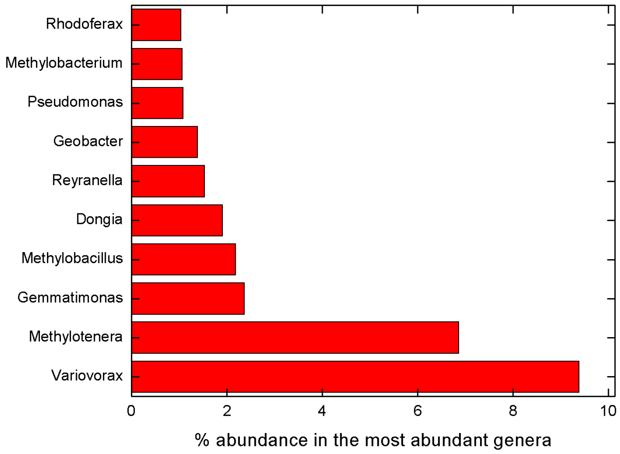
BPNB



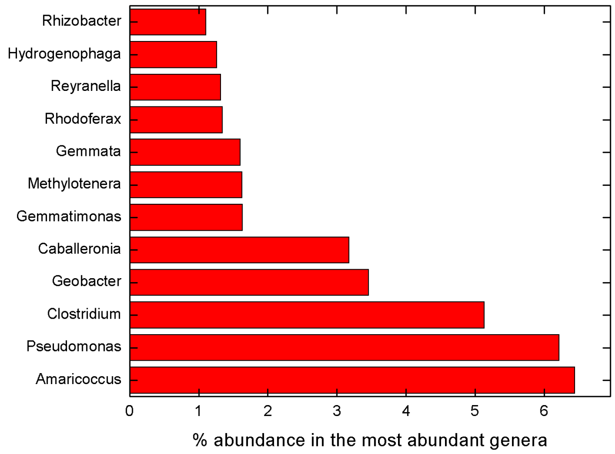
BPC



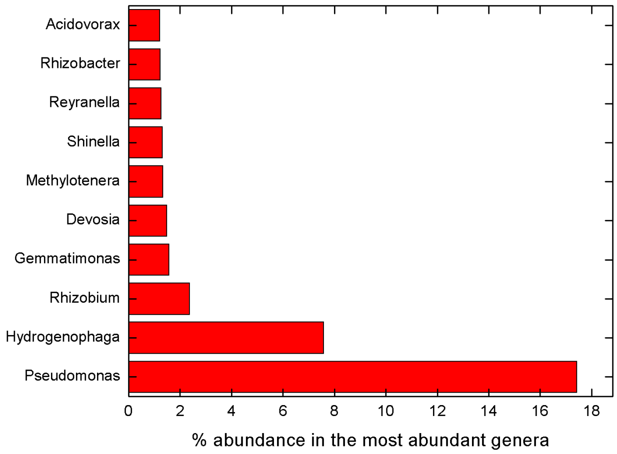
BPNC



BRA



BRB



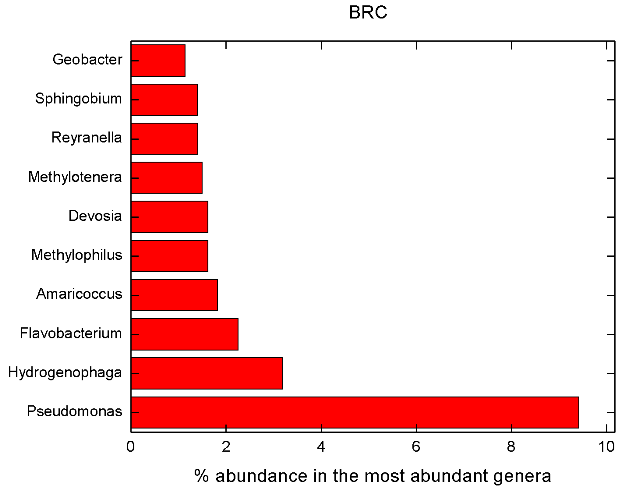


Figure 8.5. Abundance of the most abundant genera with known organic compound removal potential. BPB = Bioremediation Roots Iris, BPNB = Bioremediation New Roots Iris, BPC = Bioremediation Roots Phragmites, BPNC = Bioremediation New Roots Phragmites, BRA = Bioremediation Rhizosphere Lythrum, BRB = Bioremediation Rhizosphere Iris, and BRC = Bioremediation Rhizosphere Phragmites.

From the genera mentioned in the previous figures, the most abundant ones are shown in Table 8.3.

Table 8.3. Relative abundance of the most common genera for organic compound removal.

Sample	***Pseudomonas***	***Flavobacterium***	***Variovorax***	***Methylotenera***	***Reyranella***	***Amaricoccus***	***Hydrogenophaga***
				%			
BPB	9.39	2.22	0	1.56	1.41	1.84	3.2
BPNB	1.48	0	2.93	7.96	1.38	0	0
BPC	4.47	2.03	2.36	13.01	1.02	0	0
BPNC	1.09	0	9.39	6.86	1.54	0	0
BRA	6.22	0	0	1.62	1.31	6.44	1.26
BRB	17.43	2.61	0	1.34	1.26	0	7.58
BRC	9.42	2.26	0	1.5	1.41	1.82	3.19

We could not identify major trends for the abundance of the genera in the studied samples (Table 8.3). For example, *Flavobacterium* is present in the rhizosphere and root samples of *Iris* and *Phragmites* but not in the rhizosphere of *Lythrum*, and it is not present in the new roots. Hydrogenophaga, similar to *Amaricoccus*, are genera present in most of the rhizosphere samples but only in one root sample (*Iris*). These genera can remove a broad range of organic compounds. *Pseudomonas* is a well-known genus for organic and inorganic pollutants' removal and is commonly present in CWs [388], e.g., herbicides, antibiotics and the anticonvulsant carbamazepine [379,389,390]. *Amaricoccus*, similar to methylotrophs (in this case, *Methylotenera*), is a genus that uses organic compounds as a carbon source [391]. Some genera are targeting specific compounds; for example, halogenated compounds (diclofenac, TCIPP), as it is in the case of *Variovorax* and *Flavobacterium* [392,393].

8.3.4 Colonization of the Roots by AMF

To broaden the knowledge about a plant roots' microbiome, we observed the presence of the AMF complement by quantifying their colonization in the plant roots using microscopic techniques. AMF belong to endomycorrhizae, meaning that the hyphae penetrate individual root cells of the plant [394]. Thanks to this knowledge and to comparisons with previously made photographs of AMF, it is possible to detect the fungus' nature successfully (Figure 8.6).

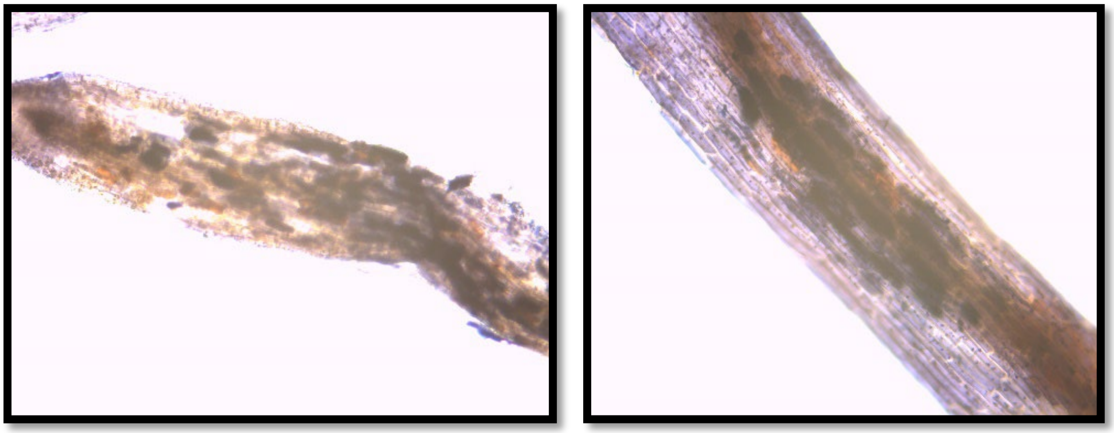


Figure 8.6. AMF in the plant cells of *Iris* (both photos represent the plant cells of *Iris*). The AMF appear as the very dark, even black spots within the plant cells.

The observed colonization rate of the roots by AMF is shown in the Table 5.4.

Table 8.4. Colonization of the plants' roots by AMF in the studied root samples.

Sample	**Colonization by AMF (%)**
Phragmites before bior. exp.	34
Iris before bior. exp.	56
Lythrum before bior. exp.	36
Phragmites after bior. exp.	10
Iris after bior. exp.	15
Lythrum after bior. exp.	10
Phragmites after bior. exp. new roots	0
Iris after bior. exp. new roots	0
Lythrum after bior. exp. new roots	0

We found that *Iris* consistently exhibited the highest AMF colonization rates. This could be due to *Iris* having a very dense root system compared to the other plants. Unfortunately, the roots suffered some damage during removal from the soil in the semi-hydroponic installation, which resulted, together with the majority of the soil absent, in an overall decreased AMF colonization. During the bioremediation experiment, we also observed fresh root growth. These examined roots showed no evidence of AMF, which may be explained by the fact that these fungi are soil-borne [229,360,395]. As the roots were not further investigated, the symbiosis between the fungi and the plants was not evaluated further in the present work. Thus, a possible target of future studies could be a deeper analysis of the roots and their associated AMF with possible extraction of the accumulated MPs.

The results acquired in this study indicated that the aforementioned genera are able to contribute to the removal of MPs when the plant roots for the symbiotic AMF are enriched, which is assumed to improve the phytoremediative potential of the plants. This confirms our hypothesis that rhizosphere in CWs has positive impact on the removal of MPs.

8.4 Conclusions

In this study, experiments determining the bioremediative activity of the studied systems for the removal of MPs were carried out. Next, the rhizosphere microbiome was identified, and the genera responsible for the removal of organic MPs were characterized. Additionally, the colonization of the plant roots by AMF, which enhanced the removal of the MPs, was determined. The conclusions of the present research are as follows:

- Compared to our previous phytoremediation experiments, the currently described bioremediation experiment in semi-hydroponic conditions showed improved MP removal, which we believe was due to the additional aeration, recirculation of the liquid medium, and commercially bought hydroponic solutions, which favor the growth conditions of the plants and, therefore, enhance the development of the rhizosphere and consequent removal of MPs.
- The most efficient bioremediative system was the system with *Iris pseudacorus*, which removed 22 out of 27 of the MPs with more than 80% efficiency.
- Compounds, which are not well-removed in other bioremediation experiments, were removed here, with more than 90% efficiency (e.g., beta-blockers, carbendazim, cyclophosphamide, and DEET).
- Generally persistent compounds were removed with high efficiency (metoprolol up to 91%, lidocaine up to 84%, and TCIPP up to 90%).
- Possible ongoing nitrification likely enhanced the bioremediative process, as many of the MPs are degraded by nitrifying bacteria.
- *Lythrum salicaria* had the lowest efficiency for removing MPs (contrary to previous phytoremediation experiments). This is probably due to its weak physiological status after the winter season.
- *Pseudomonas, Flavobacterium, Variovorax, Methylotenera, Reyranella, Amaricoccus* and *Hydrogenophaga* belong to genera that are known to be potential MP degraders. High abundances of these organisms were also found in our samples.

- A colonization of the plant roots by AMF was established. This information is valuable, as AMF contribute to phyto- and bioremediation. The macrophyte with the highest colonization was *Iris pseudacorus* (56%).

These conclusions summarize the main outcomes of the discussed research. In the present study, the optimal candidate for bioremediation was found to be *Iris pseudacorus*. It showed an excellent ability to outlast the winter season without considerable loss of its pollutant removal abilities and provided a decisive environment for its advantageous symbiosis with AMF. We believe there is much potential for further investigation of bioremediative systems, their associated microbiomes, and CWs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14073944/su14073944/s1.

Author Contributions: Conceptualization, H.B., S.V., and J.H.; methodology, H.B., S.V., and L.L.; validation, H.B., S.V., and J.H.; formal analysis, H.B. and S.V.; investigation, H.B.; resources, J.H.; data curation, H.B.; writing—original draft preparation, H.B.; writing—review and editing, H.B., S.V., C.C.L., L.L., and J.H.; visualization, H.B.; supervision, S.V. and J.H.; project administration, S.V. and J.H.; funding acquisition, S.V. and J.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

9 Closing chapters

9.1 Summary of results and conclusions

Constructed wetlands

The application of CWs in laboratory conditions showed their excellent ability to remove MPs from synthetic wastewater, resulting in average removal rates for the three best performing substrates (sand, sand and 15% activated biochar and sand and 15% zeolite) 94, 94 and 95% respectively. The three wetlands with the mentioned substrates were consequently applied for treatment of wastewater effluent of WWTP Reisdorf, resulting in highest removals of relevant compounds (diclofenac, carbamazepine, clarithromycin and benzotriazole) for catchment of river Sûre for the CW with the 15% biochar admixture (in average 99, 92, 96 and 98%, respectively. The CW with 15% biochar admixture was then, as the best performing substrate, applied at WWTP Echternach for elimination of MPs from its effluent. Also here the wetland reached required discharge limits for the relevant compounds. Overall, the CWs in VF configuration resulted to be efficient in removal of MPs complying with the local regulations for discharge.

Removal mechanisms

In order to fulfill the main aims of the PhD research, it was needed to create a concept, which would help to determine the contribution of different mechanisms present in the studied CWs for removal of MPs.

This knowledge can help immensely to foster the design and implementation of CWs as additional step for MP removal in future in regards to MPs' elimination due to these reasons:

- It is possible to determine the most effective media in wetlands, which contribute to removal of MPs.
- Application and optimization of these media offer the possibility to enhance the total efficiency of wetlands used for discharge of MPs (examples of optimization: application of a plant, which will be able to surpass well cold conditions, to up take MPs also during this period and to create and keep rich rhizosphere for the establishment of the microbial populations).

- With knowledge of the processes relevant for removal of MPs it is possible to control the efficiency of the wetland (for example, intermittent flow resulting in longer HRT (as discussed in case of adsorption) results in better removal of the MPs thanks to the established aerobic conditions and longer contact time of the MPs in the wetland media allowing the time for the treatment). The same is valid in case of aeration and recirculation – these factors proved to be beneficial for removal of MPs during bioremediation and they could also enhance the removal of MPs when generally applied in wetlands. Aerobic conditions can be established by the intermittent flow in VF configuration.

The concept has been developed in several stages:

- First, it was needed to identify the main removal mechanisms in studied VFCWs, which was achieved by comprehensive literature research.
- Second, it was required to design the experiments representing each elimination mechanism (these were identified as adsorption, phytoremediation and bioremediation).
- Last, the experiments have been performed and contribution of each mechanism to removal of MPs in vertical subsurface CWs has been evaluated.

The PhD research presented in this dissertation helped to fill in these knowledge gaps:

- *Hydraulics barriers of surface flow wetlands*

In the lab- and pilot-scale CW installations were tested different hydraulic load scenarios, starting at 100 L d^{-1} m^{-2} HLR and reaching 300 L d^{-1} m^{-2}, which is quite a high value for longer-time planned performance of CWs [120,140,396]. The main barrier of usage of the high HLR is of course clogging, which is than created due to the water blockage in the wetlands' pores. The intermittent flow confirmed to be the best option when operating the CWs under these relatively high HLRs [134,397].

- *Contaminants of concern*

Of course, one of the main roles of CWs is removal of pollutants and micropollutants and there have been many studies dedicated to this topic. However, the research done in EmiSûre and this thesis is unique in that point, that it investigated elimination of 27 compounds from different fields of applications including some very persistent compounds. What matters the most is that the wetland applications and the experimental set-ups for determination of the removal mechanisms were successful in the removal of many of these persistent compounds (e.g. carbamazepine, carbendazim, DEET, diclofenac, diuron, lidocaine, terbutryn).

- *Contribution of microbial communities to removal of MPs*

Also for this knowledge gap there have been studies, which performed characterization of these communities [398,399], however, the attempts to determine the contribution of the present microbial communities mostly include inoculation studies [400–404] or targeted spike, which are then focusing on the contribution and its optimization of the microbiome on the removal of MPs, but lack to show the role of the *naturally* present microbial communities in removal of MPs. For determination of the naturally occurring bioremediation in the CWs was developed tailored experimental set-up, where were used macrophytes with already settled microbial communities from previously established CW. This allowed to characterize and to observe the role of the present communities in the removal of MPs. Furthermore, thanks to the design optimization (additional aeration and recirculation of the reaction matrix) it was possible to enhance the bioremediative processes (e.g. continuous oxygen supply reduces stress in the rhizosphere) towards removal of the MPs. The suitability of these aspects have been already mentioned in the literature and their application in this particular experimental set-up resulting in enhanced removals confirms importance of these factors.

9.1.1 Review of the main results and conclusions

Overall, following research aims have been targeted:

Generally:

- The mechanisms contributing to the removal of MPs in VFCW have been identified (adsorption, phytoremediation and bioremediation) (Fig. 9.1.).
- Experimental set-ups for quantification of each mechanism have been developed and applied.
- Adsorption, phytoremediation and bioremediation have been quantified in removal of the studied 27 MPs.
- Role of each removal mechanism in VFCW has been assessed.
- Some of the MPs' properties have been assigned to the removal mechanisms (further in chapter 9.1.2.)
- Gaps in the experimental set-ups for quantification of each mechanism have been identified and solutions for future research have been drawn (chapter 9.2.).

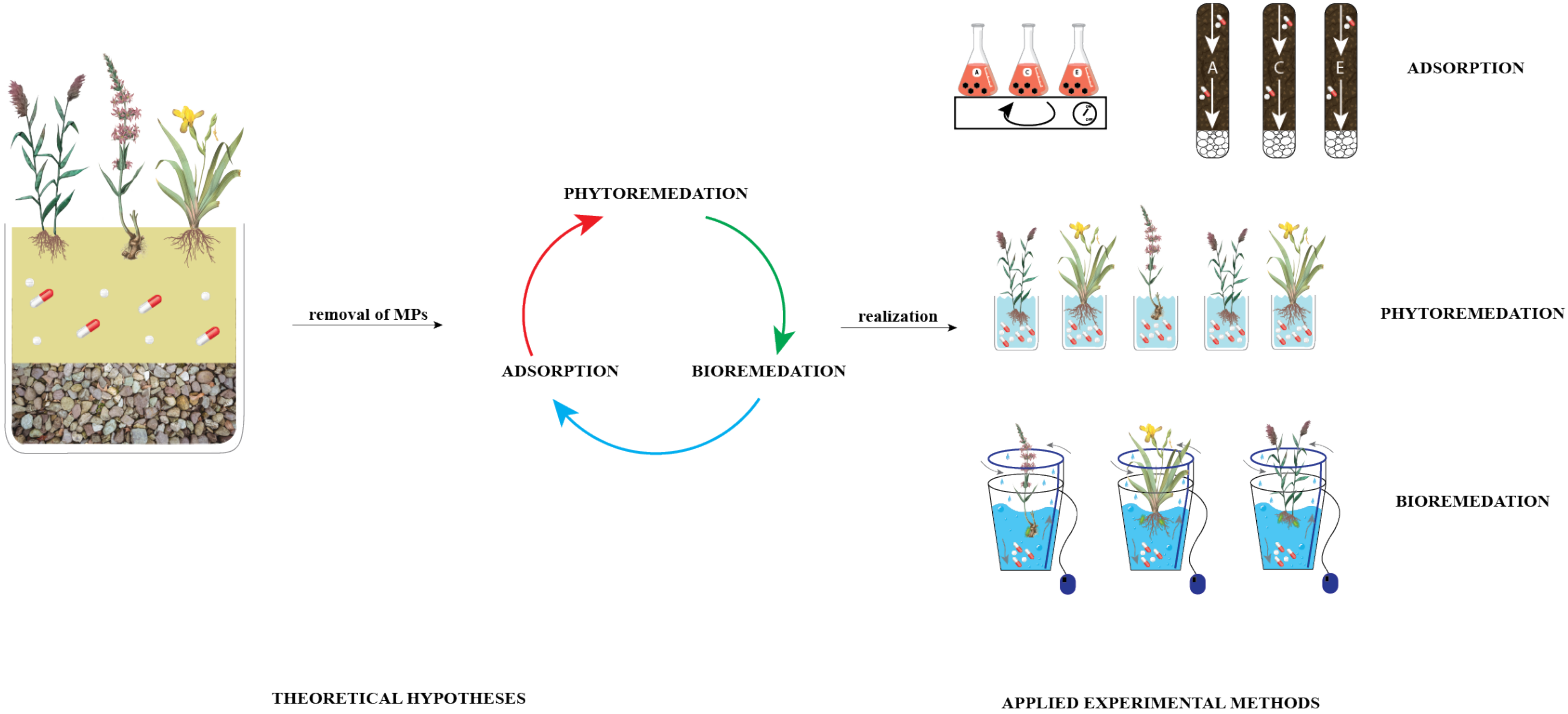

Fig. 9.1. The general hypothesis about removal mechanisms of MPs in CWs and experimental solutions of the targeted issues

Coming back to the original aims concerning the removal mechanisms, they have been targeted with help of the carried out experiments and the hypotheses have been confirmed. Overview of the aims, hypotheses and results is available in Fig. 9.2.

AIMS	HYPOTHESES	RESULTS
GENERAL		
I. establishment of the contribution of each mechanism for removal of MPs	application of specific substrate or plant may result in higher removals	mixture of bentonite sand with 15% activated biochar with *Iris pseudacorus* seem to contribute mostly to the removal of MPs
II. determination of the most significant mechanism for removal of MPs		the most significant mechanism for removal of MPs seems to be, in this case, adsorption, followed by bioremedation and phytoremedation
TARGETED		
ADSORPTION		
I. establishment of the general adsorption efficiency of the three most effective substrates	mixture of bentonite sand with 15% activated biochar might show highest adsorption ability	mixture of bentonite sand with 15% activated biochar shows generally highest adsorption ability
II. determination of the adsorption efficiency for removal of MPs of the three most effective substrates	mixture of bentonite sand with 15% activated biochar might show highest adsorption ability	mixture of bentonite sand with 15% activated biochar shows highest adsorption ability towards the studied MPs
PHYTOREMEDIATION		
I. establishment of the uptake potential of the plants for MPs	some plants may show better removal efficiency (*Lythrum salicaria* is able to create extensive root system with established AMF symbiosis, which is beneficial for phytoremediation)	the highest ability to uptake MPs showed *Lythrum salicaria*
BIOREMEDATION		
I. establishment of the contribution of the rhizosphere microbiome to the removal of MPs	certain bacterial genera might have a direct impact for the removal of MPs	7 bacterial general seem to have direct impact on the removal of MPs *(Pseudomonas, Flavobacterium, Variovorax, Methylotenera, Reyranella, Amaricoccus* and *Hydrogenophaga)*
II. identification and abundance of the present bacterial genera		present bacterial genera have been identificated and quantified
III. determination of the colonization by the arbuscular mycorrhizal fungi of the plant roots	the fungi create symbiotic relationship with the plants resulting in higher removal of MPs	a macrophyte with the highest colonization was *Iris pseudacorus* (56%), which also resulted to be the most succesful bioremediative system

Fig. 9.2. The targeted hypotheses about removal mechanisms of MPs in CWs and results obtained within this PhD work.

The content of Fig. 9.2. is discussed in detail below for each mechanism with the description of the individual research tasks:

Adsorption:

- Comparison of the studied unconventional sand-based substrates with conventionally used granulated activated carbons has been addressed during batch adsorption experiments with a dye as a proxy. It was found out that the studied substrates have substantially lower adsorption capacity. However, the adsorption capacity of these substrates in combination with the other removal processes within the wetland, especially bioremediation, is contributing to global high removal capacities of the studied MPs. Another not-to-be-forgotten fact is that the sand-based biochar used in this project is produced locally in the Greater Region – more specific in Rhineland-Palatinate - , which is an added value to the substrate's sustainability.
- The best general adsorption efficiency of the substrate C was confirmed during the experiments with the packed-bed columns, where substrate C was identified as the optimal substrate for removal of MPs as well.
- The physical and chemical properties of the unconventional sand-based substrates have been characterized. The results showed that the - for these unconventional substrates - big active surface area (20 m^2/g) and small median pore diameter (69 mm) contribute to the high efficiency of the substrate C.
- For the studied MPs have been created commonly used adsorption models and it was found out, that the best fitting model for removal of the MPs is the combined Langmuir-Freundlich model. This model allows to describe optimally adsorption characteristics of studied substrates, such as maximum adsorbed capacity, heterogeneity of the surface of the substrate, eventually could help to establish the lifetime or to design a tailored treatment.
- The parameters needed for industrial upscale of the substrate C have been specified (e.g. height of the mass transfer zone, detected in 37cm height of the bed for atenolol, breakpoint or saturation point).
- Role of adsorption within VFCW has been assessed.

Phytoremediation:

- For determination of the phytoremediation potential of the studied macrophytes, experiments in hydroponic conditions have been carried out.
- The operational parameters of the process have been investigated and optimized (e.g. additional support of the plant growth with the LED light source, proper choice of the nutrients, prolonged acclimatization period and ensured oxygen supply).
- It was found out that plants in a more mature state are able to up take higher amount of MPs.
- Results showed that the optimal macrophyte for the hydroponic assessment of phytoremediation of the studied 27 MPs is *Lythrum salicaria*.
- Role of phytoremediation within VFCW has been assessed.

Bioremediation:

- Novel experimental set-up for the assessment of bioremediation in semi-hydroponic conditions has been introduced.
- This set-up shows increased removal of MPs compared to the phytoremediation, confirming the inevitable contribution of the rhizosphere microbiome to removal of MPs - especially to removal otherwise persistent compounds (metoprolol, lidocaine, TCIPP).
- Importance and impact of operational parameters, such as recirculation of the MPs' solution and additional aeration on the MPs' removal efficiency have been stressed.
- The most effective system for bioremediation of 27 MPs has been identified – *Iris pseudacorus*
- *Lythrum salicaria,* which was in its weakest physiological status at the beginning of the experiment, was showing lowest removals in this type of experiments. It is probably due to the low adaptability of this plant to the new conditions resulting in not-well developed rhizosphere and therefore reduced field of activity for microorganisms. This shows that bioremediation is a very complex process, strongly dependent on the health of the plant (which means healthy and dense rhizosphere)

and on other factors (previously mentioned sufficient aeration, circulation of the liquid solution, but also level of nutrients).

- Bacterial genera contributing to the removal of the MPs have been found and quantified, it was found out that the most abundant genera contributing to removal of MPs in the studied samples are *Pseudomonas, Flavobacterium, Variovorax, Methylotenera, Reyranella, Amaricoccus* and *Hydrogenophaga.*
- A role and colonization the macrophytes' roots by AMF has been assessed, a macrophyte with the highest colonization was *Iris pseudacorus* (56%). This macrophyte showed also highest removals of the MPs - this fact confirms importance of the fungi in the pollutant removal processes by the plants.

9.1.2 Micropollutants and mechanisms: removal and its relation to the MPs' properties

To be able to imagine and seize contribution of each mechanism to the removal of all 27 studied MPs, Fig. 9.3 has been created. On this figure are shown medium removal efficiencies of the most efficient systems of each mechanism - sand + 15 % activated biochar for adsorption, *Lythrum s.* for phytoremediation and *Iris p*. for bioremediation. The contribution of pure bioremediation (done by the bacteria, BIOREMEDIATIONpure labeled in Fig. 9.3.) is calculated as deduction of the values for bioremediation from the values for the phytoremediation, because, in case of the bioremediation experiments were used the macrophytes with their rhizospheric system and present bacteria. Values used for creation of the Fig. 9.3. are available in Tab. 9.1. The Fig. 9.3. illustrates the role of the removal mechanisms in the overall removals within the wetland, however, for detailed discussion it is important to focus on the measured values represented by the *medium efficiency values in the left part* of the Tab. 9.1.

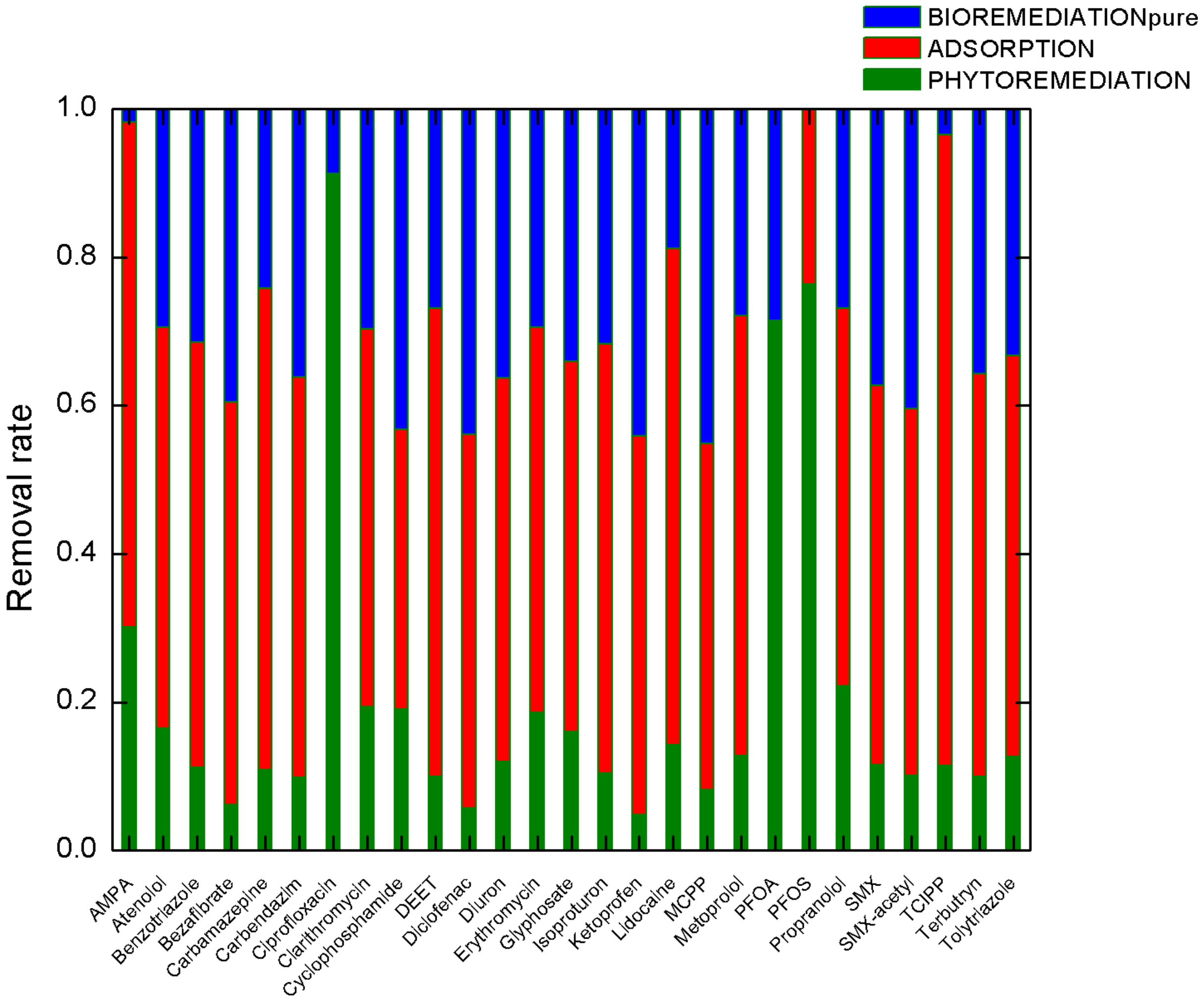

Fig. 9.3. Illustration of medium efficiency of the most efficient systems for each mechanism (sand + 15 % activated biochar for adsorption, *Lythrum s.* for phytoremediation and *Iris p.* for bioremediation), normalized.

In the Tab. 9.1. are available measured average concentrations for each mechanism obtained for the best performing system with standard deviations for each compound and from these calculated normalized values of the calculated ones (right part of the table), which create basis for the chart 9.3. In case of ciprofloxacin and PFOS for bioremediation the standard deviation is not measured due to high analytic uncertainties.

Tab. 9.1. Studied compounds with measured medium efficiencies for each mechanism ±std and consequently calculated normalized values

Compound	Medium efficiency (%)							Normalized values (%)		
	PHR*	±std	ADS*	±std	BIORpure	BIOR***	±std	PHR	ADS	BIORpure
AMPA	19.3	9.9	43.6	63.4	1.1	20.4	24.7	30	68	2
Atenolol	27.6	23.8	90.4	10.4	49.2	76.7	24.2	17	54	29
Benzotriazole	18.9	18.7	96.2	3.1	52.8	71.7	20.6	11	57	31
Bezafibrate	11.0	9.4	97.0	2.5	70.6	81.7	29.5	6	54	40
Carbamazepine	15.3	10.2	91.2	7.5	33.8	49.1	21.7	11	65	24
Carbendazim	17.7	12.4	97.4	2.4	65.1	82.8	15.3	10	54	36
Ciprofloxacin	89.7	6.9	0.0	N/A	8.5	98.2	1.01	91	0	9
Clarithromycin	32.3	19.7	85.3	10.5	49.5	81.9	20.3	19	51	30
Cyclophosphamide	16.1	18	32.0	12.7	36.6	52.7	27.2	19	38	43
DEET	12.3	16.5	77.8	13.4	33.2	45.5	42.2	10	63	27
Diclofenac	10.9	9.9	96.2	3	83.7	94.6	6.2	6	50	44
Diuron	22.5	12	97.5	2.3	68.2	90.7	12.2	12	52	36
Erythromycin	31.9	21.7	89.4	9.4	50.5	82.4	20.5	19	52	29
Glyphosate	23.4	6.7	73.1	33.4	49.7	73.0	26.9	16	50	34
Isoproturon	17.4	12.4	95.9	3.5	52.5	69.9	26.5	10	58	32
Ketoprofen	8.8	11.2	93.0	5.6	80.4	89.2	18.9	5	51	44
Lidocaine	19.0	11.9	89.5	10.4	25.0	44.0	26.8	14	67	19
MCPP	10.9	10.5	62.0	18.3	60.0	70.9	30.7	8	47	45
Metoprolol	20.2	19	93.6	5.15	43.9	64.1	23.0	13	59	28
PFOA	16.6	19.2	0.0	67.8	6.6	23.3	25.6	72	0	28
PFOS	22.3	44.3	6.9	82	0.0	0.0	N/A	76	24	0
Propranolol	42.1	20.3	96.6	3	51.0	93.1	6.9	22	51	27
Sulfamethoxazole	18.8	10.5	82.9	12.7	60.4	79.2	9.6	12	51	37
Sulfamethoxazole-acetyl	15.7	12.5	77.2	13.5	63.0	78.6	26.3	10	50	40
TCIPP	11.9	18.9	87.9	13.8	3.6	15.4	42.7	11	85	3
Terbutryn	17.5	10.1	95.8	2.9	63.0	80.5	22.6	10	54	36
Tolytriazole	22.7	14.9	97.1	2.1	59.5	82.2	15.4	13	54	33

Lythrum salicaria*, **substrate C, * *Iris pseudacorus*

Adsorption

From the Fig. 9.3. it is clear that mechanism, which is contributing predominantly to the removal of MPs is adsorption (ADS), followed by bioremediation (BIORpure) and phytoremediation (PHR).

It is intricate to set a connection between the properties of the MPs and the removal, because the variety of the compounds is broad (heterogenous, mostly aromatic compounds (except of cyclophosphamide, macrolides, glyphosate, AMPA, TCPP and fluorosurfactants)). When looking at the standard deviations available for the measured removals of the compounds, it is clear that most of the deviations is quite low, except of AMPA and glyphosate. This is given due to mutual retransformation relationship of these two compounds.

At the beginning of this dissertation it was mentioned that aromatic compounds tend to be adsorbed to organic substrates, thanks to the π-π interactions between the aromatic circles of the substrate and the compounds. This hypothesis was possible to confirm in most of the compounds, except of: ciprofloxacin, macrolides and glyphosate. Ciprofloxacin is in general an unstable compound and the low adsorption efficiency is caused due to analytical problems. The macrolides, even though non-aromatic compounds, are both containing high amount of hydrogen acceptor atoms, such as oxygen and nitrogen. Therefore, it is possible that the adsorption occurs in cases of these two compounds by a mechanism called hydrogen bonding, which is a bonding between hydrogen donors (e.g. OH groups within the structure of biochar) and acceptors (O and N in structures of macrolides) [405,406]. Another property, which is crucial for determination of the adsorption strength is the hydrophobicity or hydrophilicity characterized by the parameter log Kow, mentioned in the chapter 3.1.2.1. In general, compounds with higher log Kow, which are more hydrophobic, tend to be better adsorbed to the organic substrates. It is a case of most of the compounds, except of the fluorosurfactants, which are generally known for their desorption behavior. Interestingly, atenolol has low log Kow (0.16), however, probably in case of this compound are stronger the π-π interactions. Cyclophosphamide, which is a non-aromatic compound, but has quite low log Kow as well (0.63), is not removed well during the adsorption experiments (on average 32%). This also confirms the previous fact, as in case of atenolol, that the π-π interactions have probably in this case bigger impact on the adsorption strength, than the hydrophilicity of the compound. In the Tab. 9.2. are sorted the studied compounds according to the value of log Kow.

Tab. 9.2. Studied compounds sorted according the value of the log Kow.

Substance	**log Kow**
Erythromycin	8.9
PFOA	4.81
Diclofenac	4.51
PFOS	4.49
Bezafibrate	4.25
Terbutryn	3.74
Propranolol	3.48
Clarithromycin	3.16
MCPP	3.13
Ketoprofen	3.12
Isoproturon	2.87
Diuron	2.68
TCIPP	2.59
Carbamazepine	2.45
Lidocaine	2.26
DEET	2.02
Metoprolol	1.88
Carbendazim	1.52
Benzotriazole	1.44
Tolyltriazole	1.08
Sulfamethoxazole	0.89
Sulfamethoxazole-acetyl	0.86
Cyclophosphamide	0.63
Ciprofloxacin	0.28
Atenolol	0.16
AMPA	-1.63
Glyphosate	-3.4

In case of all compounds we can exclude covalent binding as an adsorption mechanism, because it requires that both adsorbent and adsorbent are charged. In this case, all the compounds are in their neutral state, as are the substrates, whose surfaces are not additionally charged [407].

Phytoremediation

Phytoremediation resulted to be the weakest removal mechanism, its efficiency is directly connected to plants, their health and vegetative status. Also in case of this mechanism is the coefficient log Kow an important parameter. However, in this case it is opposite as in case of adsorption; the compounds, which have lower log Kow and are therefore more hydrophilic, are tending to be better up taken by the plants, because they are better dissolved in water. Example of ciprofloxacin is confirming this fact. Fluorosurfactants are in this case removed better, or at least more stable, than in case of the other 2 mechanisms (e.g. PFOA up to 35%, as shown in publication [III]). However, this is given by their high persistency and often occurring desorption events during the adsorption. The potential of phytoremediation of fluorosurfactants is also mentioned in the literature, as shown in the properties of MPs mentioned in the Objective chapter.

Bioremediation

Bioremediation is the most complex and least predictable mechanism of all, because it depends on many factors. The bacteria responsible for the removal are mostly targeting general function groups, e.g. genera targeting halogenated compounds (as in case of *Variovorax*), general decompositors of organic matter (*Pseudomonas, Reyranella*) or benzene degradators (*Hydrogenophaga*). When looking at the standard deviations calculated for each compound's removal during the bioremediation experiments, it can be observed that the standard deviations are higher, than the ones for phytoremediation and for adsorption. This suggests that the ability of the bacteria to remove the MPs is fluctuating during the whole experiment. Compounds, such as bezafibrate, diclofenac and ketoprofen have been proven to be well biodegradable, as mentioned in the chapter Objective. AMPA, as mentioned previously, is a degradation product of glyphosate, which tends to retransform back to its parent compound, therefore removals of this compound are generally low. Fluorosurfactants or carbamazepine are very persistent compounds and they are not biodegradable [408,409]. TCIPP is also not well biodegradable due to presence of chlorine in its structure

[410]. Ciprofloxacin, as an antibiotic, inhibits the microbial activities, therefore it is resistant to bioremediation as well [411].

To summarize, the results achieved confirm that adsorption is a leading mechanism towards removal of MPs in CWs in the vertical flow subsurface configuration, followed by bioremediation and phytoremediation. It is possible that this arrangement would change in time in favor of bioremediation with increasing saturation of the substrate's surface and therefore resulting in decreasing adsorption.

9.2 Outlook and future research

With the information gained in the conducted research, it is possible to proceed in further investigations about the efficiency of the CWs and also targeting specific compounds and processes. This information is even more valuable, as some of the persistent compounds (carbamazepine, carbendazim, DEET, diclofenac, diuron, lidocaine, terbutryn), which are normally not well eliminated in other advanced wastewater treatment processes, are removed here with noticeable efficiency.

Generally, for further usage of CWs for the discussed purpose and configuration, following aspects could be taken into account:

- In the adsorption experiments were compared two types of the biochar: activated and non-activated. Activated biochar resulted in higher adsorption efficiency, suggesting its further applications in the wetland units favored over non-activated biochar.
- As the wetlands were operated also during the winter season (which can drop to -10 °C in Luxembourg), it is needed to isolate the wetlands with a layer of mulch, which was done in the pilot-scale installations. However, the mulch layer on the top of the wetland creates an unsaturated zone for the plants to root in [412]. This requires usage of plants, which are better adapted to the winter periods. As could be observed in case of *Lythrum salicaria*, this plant did not outlast the

winter period well. If the plant would be in a better physiological state, the removal efficiency of the wetland could have been enhanced. Some of these species, which are suitable for the cold climate conditions, are e.g. *Sagittaria latifolia*, *Silphium perfoliatum* or *Solidago rigida*. All these species are flowering in the summer months, and therefore contributing to biodiversity and creating beneficial surroundings for the insects.

- Longer feeding periods (e.g. in case of the CW Reisdorf were the cycles lasting in orders of minutes) in orders of tenths of minutes would resulti in longer HRT which contributes to enhanced removal of the pollutants.
- Combination of a VF wetland with a HSSF wetland in a hybrid system could result in enhanced removal efficiencies of the MPs thanks to prolonged HRT and co-existence of aerobic and anaerobic conditions suggesting the involvement of both aerobic and anaerobic bacteria in the removal of MPs and other pollutants (nitrogen) [413].
- Combination of CW with another quaternary technology (e.g. some of the AOPs) could result in decreased demands for the big surface needed for successful operation of the wetland.

Following considerations could be taken into account when focused only on the individual processes:

Adsorption

Generally, the adsorption process could be improved by implementation of the following ideas:

- The wetland experiments in lab-scale showed that additional increase of the activated biochar is not resulting in higher removals, however, this can be due to contribution of bioremediation balancing the difference in the removal of the compounds in case of the lower ratio activated admixture. Evaluation of the impact of the increased amount of activated biochar on the adsorption efficiency could be performed by experiments with higher ratios of this adsorbent. Just 15% admixture resulted in the BET area 30x higher than the one of just pure sand (pure sand 0.65 m^2/g and 15% biochar admixture 19.9 m^2/g), usage of pure biochar could result in the total BET area of

129 m^2/g (calculated thanks to the knowledge of the BET of the 15% activated biochar mixture and pure sand). However, this application would not be in favor of cost investments (price of pure sand 95€/t, price of pure biochar 600 – 1000 €/t (depending on amount needed, bigger amounts are cheaper/t than small amounts)).

- The sand contains iron, which is an important element for removal of phosphorus, thanks to the complexation of these two elements. In the 15% activated biochar is iron contained as well (in form of Fe_2O_3), so, in case of usage of the pure activated biochar the complexation of phosphorus with iron would not be missed. Also, once the biochar is activated, phosphorus is mostly fixed by the fermentation bacteria, whereas in case of non-activated biochar can occur release of phosphorus which is present from the pyrolysis.
- The adsorption of the compounds could be determined by batch experiments as well, with a specific focus on compounds, which not removed well (<80% on average).
- In the batch experiments, various conditions could be investigated, such as pH, temperature, and influence of the adsorption strength by the concentrations of both adsorbent and adsorbate.
- For the column experiments, the diameter of the columns could be increased resulting in bigger bed of the substrate, in prevention of clogging and, if that would be possible (depending on the clogging), in longer feeding periods resulting in longer EBCT/HRT which contributes to enhanced removal of the pollutants.

In case of adsorption was majority of the compounds removed very well (except of DEET, sulfamethoxazole-acetyl, glyphosate, MCPP, AMPA, cyclophosphamide, fluorosurfactants and ciprofloxacin) as shown in Tab. 9.3.

Tab. 9.3. Medium efficiency of the studied compounds in case of adsorption, sorted according to efficiency of the removal.

Compound	**Average removal (%)**
Diuron	97.5
Carbendazim	97.4
Tolytriazole	97.1
Bezafibrate	97.0
Propranolol	96.6
Diclofenac	96.2
Benzotriazole	96.2
Isoproturon	95.9
Terbutryn	95.8
Metoprolol	93.6
Ketoprofen	93.0
Carbamazepine	91.2
Atenolol	90.4
Lidocaine	89.5
Erythromycin	89.4
TCIPP	87.9
Clarithromycin	85.3
Sulfamethoxazole	82.9
DEET	77.8
Sulfamethoxazole-acetyl	77.2
Glyphosate	73.1
MCPP	62.0
AMPA	43.6
Cyclophosphamide	32.0
PFOS	6.9
Ciprofloxacin	0.0
PFOA	0.0

In this work, the focus was given on removal of the studied compounds with respect to the NBS – constructed wetlands. In general, the wetlands were able to remove most of the compounds with efficiencies higher than 90%. If a focus is given on particular compounds and their enhanced removal by adsorption, without consideration of further environmental aspects, the solutions could be following:

- For DEET, the enhanced removal by adsorption could be achieved by application of so-called sandwich filter (combination of different substrates in layers ensures increased removal of the pollutants – e.g. sand and very efficient, but costly GAC [414,415]).
- In case of AMPA and glyphosate, the removal is of course complicated thanks to many-times mentioned retransformation of AMPA to glyphosate. However, there are evidences that the general adsorption of both of these compounds can be enhanced by pH (if acidic 4-7) [416,417], which could be example of adjustment of future experiments focused on adsorption of these two compounds.
- There is an evidence, that enhanced adsorption of MCPP can be achieved with higher temperatures (10-20 °C - the reaction mixture in case of adsorption was cooled to 4 °C), therefore, in order to enhance the adsorption of MCPP, the experimental temperature during the adsorption experiments could be increased [418].
- Cyclophosphamide is not an aromatic compound, therefore it cannot be adsorbed on organic substrates thanks to π-π interaction. Usage of inorganic adsorbents, such as silica-nanocomposites, could result in enhanced adsorption of cyclophosphamide [419].
- No adsorption of ciprofloxacin was observed due to its low stability. This could be improved by storage or encapsulation of ciprofloxacin in specific media, such as specific minibags or microspheres, the adsorption could be than enhanced by combination of adsorption and different process (e.g. photodegradation) [420–423].
- In case of PFOA and PFOS the desorption is a commonly known problem. The desorption of these two compounds could be reduced by application of different substrates, such as aluminum-based compounds [424].

Phytoremediation

Phytoremediation was considered as a mechanism with the lowest potential for removal of MPs from the liquid solution. As both phytoremediation and bioremediation were carried out in similar (semi- or fully hydroponic conditions), some knowledge could be applied to both of these processes.

- What could be definitely improved in case of phytoremediation is sufficient amount of oxygen ensured by the aeration pump resulting in the circulation of the liquid solution. These both factors could enhance the phytoremediation potential immensely, as proved during the bioremediation experiments.
- Longer lightning periods (up to 12 hours) would enhance the photosynthesis and promote the plants' health.
- Based on the results of the comparison of fresh and adapted plants (in case of *Phragmites* the adapted plant showed better removals, in case of *Iris* it was the fresh one, however, *Iris* showed in general better potential for up take of the MPs than *Phragmites*), it would be favorable to use plants, which are in a more mature physiological status (e.g. during summer), but are not burdened with the pollutants. The solution could be usage of plants from natural wetlands or ponds known for their low presence of MPs, or garden centers offering mature plants.
- As mentioned earlier, the AMF play crucial role in the phytoremediation thanks to their symbiotic relationships with the plants. To enhance this symbiotic relation and to promote the plants' growth, it is recommended to implement some elements (e.g. selen) in the nutrient solution [425].
- Regarding the MPs, the primary focus could be given on those, which are known from literature to be well up taken by the plants (cyclophosphamide, terbutryn and fluorosurfactants).

Bioremediation

As mentioned in case of phytoremediation, some of the knowledge could be applied for improvement of bioremediation too.

- The circulation of the liquid medium together with additional aeration confirmed to have a significant impact on the removal of the MPs. These two factors should be present also in the future investigations, possibly combined with longer experimental periods in order to achieve higher removals thanks to the longer contact time of the pollutants with the microbes (e.g. 45 or 60 days).
- Healthy plants in a mature physiological state should be chosen for the future investigations, as healthy developed plants have rich rhizosphere containing microbes contributing to removal of the MPs.
- Further focus could be given on compounds, which are known to be removed well by bioremediation, however, in this case their removals were lower (e.g. bezafibrate (here on average 71%,), erythromycin (here on average 51%), isoproturon (here on average 43%), metoprolol (here on average 44%), sulfamethoxazole (here on average 63%), TCPP (here on average 4%).
- Removal of bezafibrate should be performed under higher temperatures (≥ 20 °C, in case of the here discussed experiments the temperatures were slightly under 20 °C), lower temperatures may have negative effect on the bacteria contributing to removal of this compound [426]. Normally, in the WWTPs the temperatures are significantly lower, which is probably also one of the reasons why is this compound not removed in the conventional treatment [427,428].
- In case of antibiotics is bioremediation an intricate degradation pathway, as antibiotics are developed to treat bacterial infections. Despite this fact, for some antibiotics is bioremediation a leading degradation mechanism (e.g. sulfamethoxazole (thanks to the instability of its isoxazole moiety) and partially also erythromycin). However, from the previously mentioned reason it is needed to perform the bioremediation experiments with inoculated bacterial strains coming from different sources (e.g. activated sludge or freshwater), which are able to degrade antibiotics [429,430].

- Similarly, bioremediation of isoproturon could be promoted in presence of some particular bacterial strains (such as the ones belonging to the family *Sphingomonadaceae*), which are known to enhance degradation of this compound [431].
- Bacteria from the same family – *Sphingomonadaceae* and additionally *Enterobacteriaceae* proved to have a positive effect on the degradation of metoprolol – experiments focused on removal of metoprolol could include inoculation studies with these two bacterial families [432].
- Inoculation studies could be included in the experiments focused on removal of TCIPP, as mentioned in the Publication [IV], genus *Sphingobium* is associated with degradation of this compound.

The conducted research broadened significantly the knowledge about removal mechanisms of MPs in VFCWs. During this research were found many new interesting insights, besides the theoretical knowledge also practical advances (new experimental designs), which can be further developed and targeted in future.

The problematics of MPs presence in water will be increasing with the growing population in the next years. Taking into account global environmental dilemmas, it is urgent to find technologies, which will not just help to solve problems they are developed for, but also contribute with their character to restoration of the nature's equilibrium. I believe, that constructed wetlands are one possible solution for the problematics of MPs emissions, with their inevitable contribution to extension of biodiversity given by their natural origin.

10 References

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