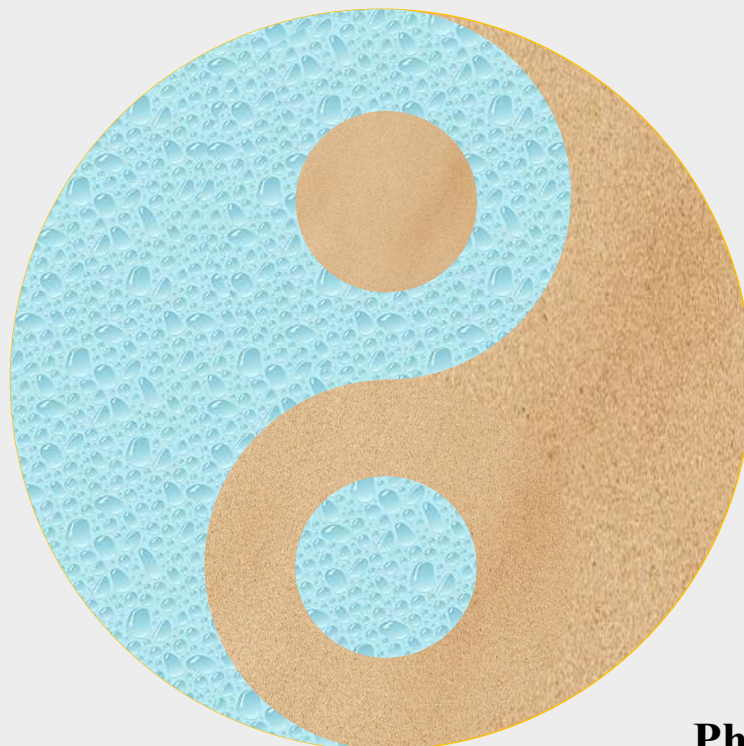


Czech University of Life Sciences Prague



**The effect of different water-saturation
levels on micropollutants removal
in constructed wetlands**



**Ph.D. Thesis
Tongxin Ren**

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Ph.D. Thesis

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The effect of different water-saturation levels on micropollutants removal in constructed wetlands

Tongxin Ren

Thesis

This thesis is submitted in fulfilment of the requirements for the Ph.D. degree at the
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The effect of different water-saturation levels on micropollutants removal in constructed wetlands

Tongxin Ren

Czech University of Life Sciences Prague (2024)

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Abstract

Nowadays, the contamination of micropollutants (MPs) in surface water and wastewater has been paid more and more attention because they are resistant to the environment and cannot be removed completely by wastewater treatment plants. Constructed wetlands (CWs) as an economic, landscape-valued and sustainable treatment method are widely used. The natural structures of constructed wetlands including substrates, water and plants constitute an advantageous environment for the removal of MPs by multiple interrelated mechanisms. Several types of CWs with different characteristics, such as different water-saturated levels and various kinds of substrates can be used to treat wastewater containing MPs. So far, the effect of water-saturation level, especially the efficiency of partially-saturated systems, on the removal of MPs in CWs has been scarcely discussed in the literature. In addition, side-by-side comparison of the MPs removal efficiency in CWs with various saturation levels is rare. Therefore, this study explored the removal of organic MPs in CWs on the lab or mesocosm scale, investigating the influence of different water-saturated levels (unsaturated, partially-saturated and saturated) and different types of substrate amendments (woodchips, iron chips and their mixture) on the degradation efficiency. The main objectives of this study were to (1) investigate the effect of different water-saturation levels in CWs on MPs removal from household wastewater; (2) evaluate the impact of the introduction of woodchips and iron chips on the MPs removal; (3) explore the effect of various saturation levels and the amendments on the removal of standard contaminants, in particular total nitrogen. This doctoral research was divided into three experiments. The experiment 1 investigated the removal of bisphenol S (BPS), fipronil (FPN) and ketoprofen (KTP) under various saturation conditions: unsaturated, partially-saturated and saturated, and mimicked the conditions occurring in unsaturated, partially-saturated intermittent vertical-flow CWs and in horizontal-flow CWs, respectively. The experimental CWs were operated BPS and KTP exhibited contrasting behaviour against FPN in the CWs. Namely, BPS and KTP were almost completely removed in the unsaturated CWs without a considerable effect of plants, but their removal in saturated CWs was only moderate (approx. 50%). The plants had only a pronounced effect on the removal of BPS in saturated systems, in which they enhanced the removal by 46%. The removal of FPN (approx. 90%) was the highest in the saturated and partially-saturated CWs, with moderate removal (66.7%) in unsaturated systems. Noteworthy, partially-saturated CWs provided high or very high removal of all three studied substances despite their contrasting degradability under saturated and unsaturated conditions. The removal of the micropollutants in partially-saturated CWs was comparable or only slightly lower than in the best treatment option making it the performance all-rounder for the compounds with contrasting biodegradability properties. The experiment 2 evaluated the removal efficiency of BPS, diclofenac (DCF), fluconazole (FCZ), ketoprofen (KTP), sulfamethoxazole (SMX), sulfanilamide (SNM) and 5-amino-3-methylisoxazole (ISX) under unsaturated CWs and saturated CWs separately in the first stage of this experiment. In the second stage, the mechanism of SMX in anoxic biofilters supplemented with iron chips and woodchips was explored. It was hypothesized that the presence of reducing conditions (additionally imposed by woodchips) and Fe (II) (from iron chips) would promote the reduction of SMX within its isoxazole cleavage. To better understand the degradation mechanisms two analogues of the SMX subunits: SNM and ISX, were also added to separate influents. The influent was a simulated household wastewater. The experimental system consisted of up-flow columns filled with sand (negative control

system), woodchips (10% v/v), iron chips (10% v/v), and mixed woodchips and iron chips (10% v/v each). The presence of iron chips (also in a mixture with woodchips) improved the removal of SMX by 68% (from 28% to 96%). The removal of SNM was negligible in all the types of columns used and, on the other hand, the removal of ISX was almost complete in all the types of treatments. Non-target LC-MS/MS analysis confirmed cleavage of the isoxazole ring within the structure of SMX. The ecotoxicity tests showed the toxicity of the influent and effluents containing SMX towards the plant *Sinapis alba*. The removal of SMX was reflected in decreased toxicity reaching a stimulative effect. On the other hand, the influent and effluents with ISX exhibited the inhibition of *Aliivibrio fischeri*. In contrast to the effect of SMX, the removal of ISX only slightly decreased the toxic effect. The experiment 3 evaluated the effect of partially-saturated and unsaturated planted constructed wetlands on the removal of 26 MPs and standard contaminants. The presence of woodchips improved the removal of 17 MPs due to sorption or providing organic carbon source for the denitrification process involved in the degradation. The use of partially-saturated CWs improved the removal of nine MPs, but the overall effect was low. The partially-saturated CWs with woodchips, however, improved the removal of total nitrogen by 46%, which qualifies these systems to be a favorable solution for the treatment of household wastewater in terms of MPs removal and TN decrease.

Overall, this study contributes to the insights into the removal efficiency of the selected twenty-eight MPs and highlights the role of partially-saturated CWs and substrates in optimizing treatment system performance.

Abstrakt

Kontaminaci mikropolutantů (MP) v povrchových a odpadních vodách je v dnešní době věnována stále větší pozornost, protože jsou perzistentní v životním prostředí a čistírny odpadních vod je nedokáží zcela odstranit. Umělé mokřady (UM) jako ekonomická, krajinně hodnotná a udržitelná metoda čištění jsou široce používány. Přirozené struktury umělých mokřadů včetně substrátů, vody a rostlin představují výhodné prostředí pro odstraňování MP prostřednictvím mnoha vzájemně souvisejících mechanismů. K čištění odpadních vod obsahujících MPs lze použít několik typů UM s různými charakteristikami, jako jsou různé úrovně nasycení vodou a různé druhy substrátů. Dosud byl v literatuře jen výjimečně diskutován vliv úrovně nasycení vodou, zejména účinnost částečně nasycených systémů, na odstraňování MP v UM. Tato studie proto zkoumala odstranění organických MP v UM v laboratorním a poloprovodním měřítku a zkoumala vliv různých úrovní nasycení filtračního materiálu vodou (nenasycené, částečně nasycené a nasycené) a různých typů úprav substrátu (dřevní štěrka, železné špony a jejich směsi) na účinnost degradace. Hlavními cíli této studie bylo (1) prozkoumat vliv různých úrovní nasycení vodou v UM na odstraňování MP z domovních odpadních vod; (2) vyhodnotit dopad použití dřevní štěrky a železných špon na odstraňování MP; 3) prozkoumat vliv různých úrovní nasycení na odstraňování standardních kontaminantů, zejména celkového dusíku. Tento výzkum byl rozdělen do tří experimentů. Experiment 1 zkoumal odstranění bisfenolu S (BPS), fipronilu (FPN) a ketoprofenu (KTP) za různých podmínek nasycení filtračního materiálu vodou: nenasyčený, částečně nasycený a nasycený, a napodoboval podmínky vyskytující se ve vertikálních umělých mokřadech. BPS a KTP vykazovaly kontrastní chování proti FPN, neboť BPS a KTP byly téměř úplně odstraněny v nenasyčených UM bez výrazného vlivu rostlin, ale jejich odstranění v nasycených UM bylo jen mírné (cca 50 %). Rostliny měly pouze výrazný vliv na odstraňování BPS v nasycených systémech, ve kterých zvýšily odstraňování o 46 %. Odstranění FPN (cca 90 %) bylo nejvyšší u nasycených a částečně nasycených UM, se středním odstraněním (66,7 %) u nenasyčených systémů. Zajímavé je, že částečně nasycené UM poskytovaly vysoké nebo velmi vysoké odstranění všech tří studovaných látek navzdory jejich rozdílné rozložitelnosti za nasycených a nenasyčených podmínek. Odstranění mikropolutantů v částečně nasycených UM bylo srovnatelné nebo jen mírně nižší než u nejlepší varianty, což je výhodné pro sloučeniny s kontrastními vlastnostmi biologické rozložitelnosti. Experiment 2 hodnotil

účinnost odstranění BPS, diklofenaku (DCF), flukonazolu (FCZ), ketoprofenu (KTP), sulfamethoxazolu (SMX), sulfanilamidu (SNM) a 5-amino-3-methylisoxazolu (ISX) v nenasycených a nasycených UM samostatně v první fázi tohoto experimentu. Ve druhé etapě byl zkoumán mechanismus odstraňování SMX v anoxických biofiltrech doplněných o železné špony a dřevní štěpku. Byla vyslovena hypotéza, že přítomnost redukčních podmínek (dodatečně vyvolaných dřevní štěpkou) a Fe (II) (ze železných špon) by podpořila redukci SMX v rámci jeho isoxazolového štěpení. Pro lepší pochopení degradačních mechanismů byly k samostatným přítokům přidány také dva analogy podjednotek SMX: SNM a ISX. Přítokem byla simulovaná domovní odpadní voda. Experimentální systém sestával z kolon s protiproudem naplněnými pískem (negativní kontrolní systém), dřevní štěpkou (10 % obj./obj.), železnými šponami (10 % obj./obj.) a směsí dřevní štěpky a železných špon (každý 10 % obj./obj.). Přítomnost železných špon (také ve směsi s dřevní štěpkou) zlepšila odstranění SMX o 68 % (z 28 % na 96 %). Odstranění SNM bylo zanedbatelné u všech typů použitých kolon a na druhé straně odstranění ISX bylo téměř úplné u všech typů ošetření. Necílová LC-MS/MS analýza potvrdila štěpení isoxazolového kruhu ve struktuře SMX. Testy ekotoxicity prokázaly toxicitu přítoku a odpadních vod obsahujících SMX vůči semenům hořčice (*Sinapis alba*). Odstranění SMX se odrazilo ve snížené toxicitě dosahující stimulačního účinku. Na druhé straně přítok a odpadní vody s ISX vykazovaly inhibici bakterie *Aliivibrio fischeri*. Na rozdíl od účinku SMX, odstranění ISX jen mírně snížilo toxický účinek. Experiment 3 hodnotil vliv částečně nasycených a nenasycených umělých mokřadů na odstranění 26 MPs a standardních kontaminantů. Přítomnost dřevní štěpky zlepšila odstranění 17 MP v důsledku sorpce nebo poskytnutí zdroje organického uhlíku pro proces denitrifikace, který je součástí degradace. Použití částečně nasycených UM zlepšilo odstranění devíti MP, ale celkový efekt byl nízký. Částečně nasycené UM se štěpkou však zlepšily odstraňování celkového dusíku o 46 %, což kvalifikuje tyto systémy jako příznivé řešení pro čištění odpadních vod z domácností z hlediska odstraňování MP a snížení koncentrace TN. Celkově tato studie přispívá k pochopení účinnosti odstraňování vybraných dvaceti osmi MP a zdůrazňuje roli částečně nasycených UM a substrátů při optimalizaci výkonu systému čištění.

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List of abbreviations and acronyms

5-MeBT: 5-methyl benzotriazole
APAP: acetaminophen
ASF: acesulfame
BPS: bisphenol S
BTA: benzotriazole
CAP: chloramphenicol
CBZ: climbazole
CFE: caffeine
CLM: cyclamate
CWs: constructed wetlands
d: day
DC: dissolved carbon
DCF: diclofenac
DEET: N, N-Diethyl-3-methylbenzamide
DIC: dissolved inorganic carbon
DOC: dissolved organic carbon
FCZ: fluconazole
FPN: fipronil
FUR: furosemide
GFRZ: gemfibrozil
HCTZ: hydrochlorothiazide
HF-CWs: horizontal flow constructed wetlands
HF-HF-VF-HF-CWs:
HF-SF-CWs: horizontal flow-surface flow constructed wetlands
HF-VF-CWs: horizontal flow-vertical flow constructed wetlands
horizontal flow-horizontal flow- vertical flow-horizontal flow constructed wetlands
HRT: hydraulic retention time
IBP: ibuprofen
IC: inorganic carbon
ISX: 5-amino-3-methylisoxazole
KTP: ketoprofen
MAD: median absolute deviation
MEP: methylparaben
MPs: micropollutants
MTF: metformin
MTP: metoprolol
OBZ: oxybenzone
ODV: o-desmethyl venlafaxine
PO₄³⁻: phosphate
pSat-uVeg: partially-saturated columns without vegetation
pSat-Veg: partially-saturated columns with vegetation
pSat: partially-saturated columns
SAC: saccharin
Sat- uVeg: saturated columns without vegetation
Sat-CTRL: saturated columns with sand
Sat-Fe-Wch: saturated columns with sand, iron chips and woodchips
Sat-Fe: saturated columns with sand and iron chips
Sat-Veg: saturated columns with vegetation

Sat-WCh: saturated columns with sand and woodchips
SF-CWs: surface flow constructed wetlands
SF-HF-CWs: surface flow- horizontal flow constructed wetlands
SMX: sulfamethoxazole
SNM: sulfonamide
SO₄²⁻: sulfate
SPE: solid phase extraction
SSF-CWs: subsurface flow constructed wetlands
SUC: sucralose
TC: total carbon
TCS: triclosan
TN: total nitrogen a total
TOC: total organic carbon
TP: transformation products
uSat-uVeg: unsaturated columns without vegetation
uSat-Veg: unsaturated columns with vegetation
uSat: unsaturated columns
VF-CWs: vertical flow constructed wetlands
VF-HF-SF-CWs: vertical flow-horizontal flow-surface flow constructed wetlands
VF-HF-CWs: vertical flow-horizontal flow constructed wetlands

1. Introduction

Numerous micropollutants were found in aquatic environment, like in surface water which is usually a source of drinking water. Micropollutants include a wide range of different compounds, as well as their metabolites and subproducts such as pharmaceuticals and personal care Products, antifungal agents, endocrine disrupting compounds, pesticides, antibiotics, food additives, surfactants, polycyclic aromatic hydrocarbons and flame retardants (Anand et al., 2022; Daughton, 2010; Rimayi et al., 2019). These micropollutants, are from industries, agriculture runoff, hospital outflow, livestock and domestic wastewater (Pal et al., 2014). As a result, micropollutants are common in the surface water from wastewater of industrial and domestic. In Europe, over 100,000 compounds find widespread application in domestic, industrial, and agricultural applications (European Chemicals Agency, 2021). The diverse origins of production, usage patterns, and disposal methods have resulted in the ubiquitous presence of these compounds in aquatic environments (Hejna et al., 2022). While micropollutants in the surface water are often present at low concentrations, ranging from several $\text{pg}\cdot\text{L}^{-1}$ to hundreds $\mu\text{g}\cdot\text{L}^{-1}$, this is sufficient to pose a significant threat to exposed ecosystems or organisms, particularly given the persistence exhibited by some of these substances (Luo et al., 2014; Wilkinson et al., 2022). There are concentrations of some selected micropollutants in various water bodies in Table 1.

Conventional wastewater treatment plants were reported not to remove micropollutants completely ideally (Tijani et al., 2013). There are three main kinds of methods of removing micropollutants which are physical methods, biological methods and chemical methods. Physical methods include adsorption by granular activated carbon and membrane separation technologies. Biological methods include biodegradation, microbial transformation and microbial/plant assimilation processes (Biswal and Balasubramanian, 2022). Regarding chemical treatment methods, there are advanced chemical/oxidation technologies and plant assimilation, hydrogen peroxide and chlorine (Gorito et al., 2017; Petrovic, 2003; Sudhakaran et al., 2013). Hence, there is an urgent necessity to advance wastewater treatment technologies for more effective prevention of the release of micropollutants into ecosystems. Constructed wetlands represent a combined approach for the removal of micropollutants, employing a blend of physical and chemical processes, characterized by their cost-effectiveness and landscape-enhancing functions.

Table 1. The concentrations of the commonly detected industrial chemicals in various water bodies.

Category	Micropollutants	Water type	Concentration range (ng/L)	Reference
Pharmaceuticals	Acetaminophen	Surface water	4.1-3422	(Jakimska et al., 2014)
	Furosemide	Raw wastewater	Up to 3372.5	(Jakimska et al., 2014)
	Metformin	Raw wastewater	Up to 16,7907	(Kot-Wasik et al., 2016)
	Hydrochlorothiazide	Raw wastewater	Up to 5072.3	(Ślósarczyk et al., 2021)
	O-desmethyl venlafaxine	Secondary wastewater	Up to 1330	(Kosma et al., 2019)
	Metoprolol	Secondary wastewater	745–5000	(Meyer et al., 2016)
	Gemfibrozil	Ground water	12-574	(Postigo et al., 2010)
	Ketoprofen	Surface water	10-109	(Lin et al., 2008)
	Diclofenac	Surface water	0.8-1043	(Stasinakis et al., 2009)
	Ibuprofen	Surface water	0.3-100	(Kasprzyk-Hordern et al., 2009)
	Sulfamethoxazole	Surface water	0.2-284	(Spongberg et al., 2011)
	Chloramphenicol	Surface water	Up to 226	(Nguyen et al., 2022)
	Fluconazole	Surface water	6-24	(Chițescu et al., 2021)
Ingredients of personal care products	Triclosan	Surface water	35-1023	(Peng et al., 2008)
	DEET	Secondary wastewater	30-5710	(Alidina et al., 2014)
	Climbazole	Surface water	2.78-278	(Chițescu et al., 2021)
	Oxybenzone	Secondary wastewater	30-380	(Alidina et al., 2014)
	Methylparaben	Raw wastewater	Up to 40,8986	(Styszko et al., 2021a)
Industrial and household chemicals	Bisphenol S	Secondary wastewater	Up to 109	(L.-H. Wu et al., 2018)
	Benzotriazole	Groundwater	Up to 180	(Dragon et al., 2018)
	5-methyl benzotriazole	Secondary wastewater	Up to 21	(Alotaibi et al., 2015)
Pesticides	Fipronil	Surface water	Up to 5.5	(Brennan et al., 2009a)
Artificial Sweeteners	Sucralose	Secondary wastewater	20-3,6000	(Brennan et al., 2009)
	Saccharin	Surface water	Up to 360	(Dragon et al., 2018)
	Acesulfame	Secondary wastewater	30-4270	(Alidina et al., 2014)
	Cyclamate	Surface water	Up to 4600	(Zirlewagen et al., 2016)
Stimulants	Caffeine	Surface water	1-1813	(Lin et al., 2008)

2. Constructed wetlands

Constructed wetlands (CWs) have been used as wastewater treatment for over sixty years (Stefanakis and Tsihrintzis, 2012; Vymazal, 2011, 2009). The complex processes of removing micropollutants in CWs include sorption, adsorption, volatilization, filtration, plant uptake, photodegradation and biodegradation, which may occur simultaneously for the degradation (García et al., 2010; Imfeld et al., 2009; Zhang et al., 2014). The mechanisms of micropollutants removal are also related to the designs of CWs such as plant types, supporting matrices, the directions of inflow (water-saturated levels) (“Constructed wetlands in Europe,” 1995; Stefanakis and Tsihrintzis, 2012; Zhang et al., 2014). The performance of CWs depends on different parameters. For example, photolytic degradation and volatilization are influenced by temperature variation, light intensity variation by water turbidity and light absorption pattern exhibited by the pharmaceutical compound (Buser et al., 1998; Klavarioti et al., 2009). The processes of plant uptake and biodegradation are related to plant species. Micropollutants can be transferred partially or completely to low-level toxic or no toxic compounds in plant issues. Some of the micropollutants can even be mineralized eventually (Wild et al., 2005; Zhang et al., 2014). Sorption, adsorption and filtration are closely related to solid matrices and hydraulic retention time in CWs (Matamoros and Bayona, 2006). CWs are typically categorized into three types: surface flow constructed wetlands (SF-CWs), subsurface flow constructed wetlands (SSF-CWs) and hybrid systems according to the wetlands hydrology (Vymazal, 2011). SF-CWs and SSF-CWs, where different plant growth forms are depicted in the case of SF-CWs (Vymazal, 2011, 2007). Hybrid systems, combining various types of CWs, may also be employed to enhance removal efficiencies (Zhang et al., 2014). The choice of configuration should align with the characteristics of the contaminants targeted for removal.

2.1. Types of constructed wetlands

2.1.1. Constructed wetlands with surface flow

CWs with the surface flow are CWs with free water surface. Open water and floating, submerged, and emergent plants are important combined elements (Vymazal, 2011). Surface Flow Constructed Wetlands (SF-CWs) closely mimic natural wetlands, featuring a shallow flow of wastewater over substrates. The hydraulic retention time (HRT) is a key parameter frequently used to evaluate the treatment efficiency of SF-CWs. It plays a significant role in determining the pollutant removal efficiency (Kadlec, 1994). The flow moves slowly along the bottom due to resistance, detours around thick plant clumps, and encounters other obstacles (Werner and Kadlec, 2000). Notably, the shortest residence time occurs in the surface layer of the micro-channels, where there is a higher degree of preferential flow. Conversely, the longest residence time is associated with water movement near drag-inducing surfaces at the wetland bottom (Werner and Kadlec, 2000). The complexity of HRT is further heightened in SF-CWs due to vertical stratification and the presence of microenvironments surrounding plant roots (Reddy and D'angelo, 1994). SF-CWs have been employed for several decades and have demonstrated success in treating agricultural drainage water because of their effective oxygen enrichment capacity (Jia et al., 2014). The primary physicochemical processes responsible for nitrogen retention in SF-CWs encompass sedimentation, ammonia adsorption, and ammonia volatilization. Additionally, key biological processes contribute significantly to nitrogen retention, including nitrogen assimilation in plant and microbial biomass, as well as biodegradation facilitated by ammonification, nitrification, denitrification, and anaerobic ammonia oxidation (ANAMMOX) (Lee et al., 2009). These combined processes make SF-CWs a robust and efficient means of retaining nitrogen in wastewater treatment and environmental conservation contexts (Johnston, 1991). Its use helps provide oxygen enrichment, mitigating the risk of hypoxia resulting from the direct discharge of municipal wastewater. Hypoxia poses a threat to native water organisms and contributes to habitat destruction. Therefore, the application of SF-CWs in this context serves as a valuable strategy to enhance the treatment process and protect aquatic ecosystems (Jenkins and Greenway, 2005).

2.1.2. Constructed wetlands with subsurface flow

In Subsurface Flow (SSF) systems, wastewater permeates through the substrates, and depending on the flow direction, SSF-CWs can be categorized into horizontal flow constructed wetlands (HF-CWs) and vertical flow constructed wetlands (VF-CWs). In HF-CWs, wastewater horizontally traverses the substrates beneath the surface of the bed planted with macrophytes. The absence of oxygen in HF-CWs promotes bacterial decomposition of organics under anaerobic conditions and facilitates the denitrification process ((Brix, 1990; Vymazal and Kröpfelová, 2009). The nitrification process was limited because of the decreasing oxidation of ammonia (Vymazal, 2011). On the other hand, in VF-CWs, wastewater flows longitudinally from the surface to the bottom of the bed planted with macrophytes. Intermittent bed draining allows air to replenish the bed. This feeding approach facilitates efficient oxygen transfer within the substrate, leading to excellent treatment performance, particularly for organic matter and nitrogen (Brix and Arias, 2005; Hazra and Durso, 2022). The adoption of VF-CWs gained significant popularity in Europe during the 1990s. This surge in popularity was attributed to the implementation of stringent nitrogen disposal limits, which favoured the use of VF-CWs over horizontal systems due to their superior capability to oxidize ammonia nitrogen (Vymazal, 2011). In VF-CWs systems, wastewater periodically fills the wetland matrix and then drains completely under the influence of gravity. Additionally, this periodic filling and draining help prevent substrate clogging. However, the removal efficiency of phosphorus removal is relatively lower than other types of CWs (Gikas et al., 2011). Based on the remarkable functions mentioned above, VF-CWs have been used in treating landfill leachates and food processing effluents with high-concentration ammonia wastewater (Wood et al., 2007). While denitrification is relatively limited in VF-CWs. To achieve higher contaminant removal efficiency, especially for total nitrogen and phosphate, hybrid systems combining various CWs types may be applied.

2.1.3. Hybrid constructed wetlands

Combining various types of CWs can be an effective strategy to achieve higher removal efficiency, particularly for nitrogen (Vymazal, 2011). The VF-CWs are designed to achieve the removal of organics and suspended solids, along with providing nitrification. On the other hand, the HF-CWs focus on denitrification, along with additional removal of organics and suspended solids. These wetland configurations complement each other in their specific functions,

contributing to a comprehensive treatment approach for wastewater by addressing various contaminants through different processes (Vymazal, 2011). By integrating the capabilities of different wetland types, VF-HF-CWs or HF-VF-CWs are both used for different enhanced aims for different types of wastewater, such as municipal wastewater (Vymazal, 2007) and hospital wastewater (“Two-stage constructed wetland for treating hospital wastewater in Nepal,” 1999). Besides VF–HF and HF–VF CWs, HF-SF CWs and SF-HF CWs were also reported to treat industrial wastewater in China (Wang et al., 1994), winery wastewater in Italy (Masi et al., 2002), sewage in Kenya (“Combination of a well-functioning constructed wetland with a pleasing landscape design in Nairobi, Kenya,” 1999) and landfill leachate in Norway (Mæhlum et al., 2018). Hybrid CWs with more than two stages, such as VF-HF-SF-CWs and HF-HF-VF-HF-CWs were also used to treat municipal sewage in Italy and Poland (Vymazal, 2013).

2.2. Removal of micropollutants in constructed wetlands

Recently, CWs have gained increasing attention due to their cost-effectiveness and impressive efficacy in micropollutants (MPs) removal (Reyes Contreras et al., 2019). Traditional wastewater treatment plants are often reported as being unable to eliminate MPs. Therefore, the consideration of alternative methods for wastewater treatment becomes imperative. CWs, as a nature-based method of wastewater treatment, characterized by low cost, landscape value, and a higher potential for MPs removal, have received increasing attention (Matamoros et al., 2008; Venditti et al., 2022). Due to the different designs of CWs, like different types of substrates, different hydraulic retention times and the presence of plants, there are various removal efficacies in the MPs. The oxygen transfer capacity is a crucial factor in MPs removal, significantly affecting the biodegradation process by cultivating diverse microorganism community structures (Ávila et al., 2014). Unsaturated vertical CWs facilitate aerobic bacteria-mediated degradation, whereas saturated horizontal CWs are conducive to reactions requiring anoxic conditions (Li et al., 2014). In this context, it is crucial to explore partially-saturated CWs, which combine aspects of both unsaturated and saturated CWs which can improve wastewater treatment alternating oxic and anoxic conditions (Grandclément et al., 2017). Notably, research on the removal of MPs from partially-saturated CWs is currently limited.

MPs primarily originate from sewage, encompassing household, medical, and various other wastewater types generated by human activities. These micropollutants often exhibit resistance to removal during wastewater treatment processes (Liu and Wong, 2013). Pharmaceutical and personal care products (PPCPs), pesticides, insecticides, and antibiotics are all detected in wastewater (Vidal et al., 2007). Global consumption of PPCPs surpasses 10,000 tons annually (Wilkinson et al., 2017). PPCPs are acknowledged as pseudo-persistent organic pollutants, garnering significant attention for their removal from the environment (Hu et al., 2021). For example, antibiotics have been extensively employed in livestock farms for both therapy and as growth promoters (Choi et al., 2016). Substantial quantities of non-steroidal anti-inflammatory drugs (NSAIDs) are gradually being used on a daily basis. This trend is attributed to the growing global population and the continuous development of new drugs with enhanced therapeutic efficacy (Mlunguza et al., 2019). Pesticides and insecticides are used in agriculture widely all over the world (Singh and Walker, 2006). The extensive application of pesticides in agricultural and urban areas can lead to the introduction of pesticides into the body through both diffuse and point sources. However, the primary processes for these introductions are often

diffuse pathways such as surface runoff, erosion, spray-drift, and leaching (Reichenberger et al., 2007). The concentration of MPs was reported in $\mu\text{g L}^{-1}$ and ng L^{-1} levels all over the world (Boyd et al., 2004; Carballa et al., 2004; Lindqvist et al., 2005; Nakada et al., 2006). MPs removal is an urgent issue long-term needs to be solved due to the potential threat to aquatic organisms and human health (Ren et al., 2023; Sossalla et al., 2021). Currently, discharge limits for MPs are not restricted by any legal. However, there are certain related regulations, exemplified by the European Decision 2015/495/EU dated 20 March 2015 (amending previous legislation), like PPCPs, antibiotics, and pesticides (Hartl et al., 2021). An increasing number of European countries have initiated preparations for forthcoming decisions by implementing national and local requirements because EU member states monitor emerging contaminants (Brunhoferova et al., 2021). For instance, Germany has made strides in upgrading 30 wastewater treatment plants (WWTPs) for the elimination of microplastics (MPs), with North Rhine-Westphalia and Baden-Wuerttemberg leading the way. The Swiss government has identified 100 out of the 700 WWTPs to receive support for an additional treatment step, aiming to achieve an average removal rate of 80% for selected MPs (Kadlec and Wallace, 2008). Plants in CWs were reported to uptake MPs well in CWs such as *Iris pseudacorus* (Stevens and Peterson, 2007). *Iris pseudacorus* was planted in all columns in this study to enhance MPs removal efficiency.

There is a different content of oxygen depending on CWs types. Hence, various nitrogen removal processes occur in saturated CWs and unsaturated CWs. Saturated CWs have a good function of nitrification but limited denitrification while unsaturated CWs exhibit a contrasting characteristic (Vymazal, 2007). Unsaturated CWs can offer the ideal space for nitrogen because abundant oxygen and saturated CWs are beneficial for denitrification under an anoxic situation (Platzer, 1999; Vymazal, 2007). The remaining difficulty of nitrogen removal is the reverse condition between nitrification and denitrification. Dissolved oxygen is demanded in the nitrification process but it would consume organic carbon which is essential for denitrification. The influence of pH can be crucial for the nitrification process (Brenzinger et al., 2015). Ammonia oxidizers can be the electron donor in the denitrification process with hydrogen or organic matter under oxygen-limited conditions (Bock et al., 1995; Goreau et al., 1980; Kuai and Verstraete, 1998). However, the competition of chemical oxygen demand (COD) for electron acceptors, along with the inadequate availability of electron acceptors during the nitrification process and the absence of a carbon source in the denitrification zone, emerges as the primary constraint on denitrification (Yuan et al., 2020). To solve this issue, the addition of

an external carbon source as supplementary was considered to provide more COD for nitrification and electron donors which can be supportive in the denitrification process.

Nevertheless, there are limited studies on nitrogen removal and micropollutant removal in unsaturated and partially-saturated CWs (Saeed and Sun, 2017). Partially-saturated columns can improve denitrification without affecting nitrification very much but denitrification cannot be enhanced well because of the limited carbon source. The incorporation of a partially-saturated bed within a single CW unit aimed to facilitate a sequential nitrification-denitrification process. In this case, woodchips as the carbon source were added to some of the columns to supply this kind of shortage. (Ren et al., 2023) reported an optimal removal of specific organic micropollutants, including bisphenol S (BPS), fipronil (FPN), and ketoprofen (KTP), using partially-saturated CWs. The effect of woodchips as external carbon sources and different saturated levels on nitrogen removal and micropollutants removal were evaluated in this study. Woodchips were also an improvement for partially-saturated CWs systems according to our previous study (Ren et al., 2023).

There is abundant literature regarding the degradation of organic micropollutants in CWs used for the treatment of household or municipal wastewater (Vymazal et al., 2017)(Hijosa-Valsero et al., 2016; Matamoros et al., 2007a; Thomas et al., 2017; Y. Wang et al., 2019a). It is widely known that household wastewater is regarded as a significant pollution source of organic micropollutants and that on-site treatment such as CWs could reduce the discharge of some organic micropollutants into the environment (Carey and Migliaccio, 2009; Daughton and Ternes, 1999). The concentrations of micropollutants in natural waters are relatively low (ng/L and µg/L levels), but still being able to affect human health and aquatic living organisms (Ávila et al., 2017). The data accumulated over two decades of research regarding organic micropollutants removal in CWs allow indicating commonly studied substances such as some members of the group of non-steroidal anti-inflammatory drugs (such as DCF) or antibiotics (such as SMX). The removal of the compounds in CWs can be highly effective when optimum conditions are provided (Li et al., 2013). However, still numerous environmentally relevant compounds have been presented in a very narrow context or were only scarcely discussed. Even for some widely studied compounds, the lack of parallel experiments under comparable conditions does not allow for drawing meaningful conclusions on their behaviour in CWs. An example of such a compound is KTP, which was discussed in at least 27 papers considering its removal in CWs (“ketoprofen” and “constructed wetlands” on the Web of Science, 15 March

2022), but the findings regarding its removal in CWs are still inconclusive. The removal of KTP was reported to be in a broad range of -31% to almost complete removal regardless of the type of a CW and a saturation level (the selected most relevant outcomes regarding KTP removal in CWs are listed in Table 2. In our previous investigations, we observed a significant enhancement in the removal efficiency of various pharmaceutical contaminants, including BPS, diclofenac (DFC), fluconazole (FCZ), KTP, sulfamethoxazole (SMX), and sulfanilamide (SNM), under aerobic conditions facilitated by manganese oxides and electron shuttles, achieving removal rates of up to 98% (Sochacki et al., 2021a, 2018). However, the removal of SMX under anoxic conditions, particularly in the presence of woodchips as a source of organic carbon and iron chips as reductive and oxidative agents, remains unexplored. There is a study about the potential reactivity of Fe (II) with SMX's isoxazole ring in soil microcosms (Mohatt et al., 2011). Nevertheless, there is no conclusive results for water-saturated systems have been reported so far. This research aims to fill this knowledge gap by investigating the removal of SMX under anoxic conditions with the unique combination of woodchips and iron chips. Our hypothesis posits that SMX can undergo faster degradation in the presence of iron chips, specifically, Fe (II), and that 5-amino-3-methylisoxazole (ISX) and SMX can serve as indicators of the site of molecule cleavage. We anticipate that the cleavage would predominantly occur in the ISX ring. This study contributes to advancing our understanding of SMX, SNM and ISX removal processes under different water-saturated levels and provides valuable insights into the potential use of specific indicators for monitoring cleavage sites

Table 2. Performance of constructed wetlands for the removal of ketoprofen.

Reference	Size	Influent water	Removal efficiency	Saturation level	Type of CW systems	Season	Temperature	Plants	Type of plants	Notable finding
(Matamoros et al., 2009)	Small-scale	Domestic wastewater	<50%	Sat& uSat	HF-VF	Summer	-	Yes	Floating or emergent, rooted wetland vegetation.	Ketoprofen is a highly recalcitrant compound to biodegradation but it is rapidly photodegraded.
(M. Hijosa-Valsero et al., 2011)	Mesocosm-scale	Urban wastewater	30% and 22%	Sat& pSat	FWS-HF	Summer& winter	-	Both	<i>Typha</i> & <i>Phragmites australis</i>	Floating macrophyte systems were useful for the removal of ketoprofen.
(María Hijosa-Valsero et al., 2011)	Mesocosm-scale	Municipal wastewater	0% to 37±32%	Sat	HF	Summer& winter	-	Yes	<i>P. australis</i>	Ketoprofen is photodegradable and is not affected by seasonal changes.
(Reyes-Contreras et al., 2012)	Mesocosm-scale	Urban wastewater	47–81%	Sat	FWS-HF	Summer& winter	-	No	<i>T. angustifolia</i> & <i>P. australis</i>	Photodegradation could be involved in ketoprofen removal. Plants become lush and hinder the entrance of sunlight.

(Carranza-Diaz et al., 2014)	Pilot-scale	Municipal wastewater	<30%	Sat& p-Sat	HF	The whole year	-	Both	<i>Phragmites australis</i>	Photodegradation can be excluded from our study due to the subsurface flow. Ketoprofen was found that it was followed an aerobic metabolic transformation route. Sulfide and redox conditions may control the removal extent of ketoprofen.
(Zhang et al., 2012)	Mesocosm-scale	Synthetic wastewater	>85%	Sat	HF	The whole year	23-32 °C	Both	Cattail (<i>T. angustifolia</i>)	Temperatures in the tropical wetlands may play a role in the higher removal efficiencies.
(Zhang et al., 2018)	Small-scale	Synthetic wastewater	51.1%–91.3%, 47.9–60.6% in summer, autumn, and winter, respectively	Combined	FWS, HF, VF	The whole year	15.2-29.8°C	Both	<i>Canna indica L</i>	SF CW without plant was the best removing KTP, the lowest was in VF CW without plant in all seasons.
(Francini et al., 2018)	Pilot-scale	Municipal wastewater	0 to >80%	Batch	Batch	Summer	-	Yes	<i>Phragmites australis</i> and <i>Salix matsudana</i>	<i>S. matsudana</i> preferentially uptook KTP than <i>P. australis</i> (which tended to uptake diclofenac)
(Llorens et al., 2009)	Full-scale	Secondary effluent of WWTP	>90%	Sat	FWS	The whole year	5-25 °C	Yes	<i>Phragmites australis</i> and <i>Typha latifolia</i>	KTP was well-removed due to high HRT and sunlight exposure

(Nuel et al., 2018)	Full-scale	Municipality (complementary treatment)	0 to >70%	Sat	FWS	The whole year	-	Yes	<i>Salix alba</i> , <i>Iris pseudacorus</i> , <i>Juncus effusus</i> , <i>Callitriche palustris</i> , <i>Carex caryophylla</i>	KTP removal performed well in summer than winter
(Zhang et al., 2015)	Mesocosm-scale	Synthetic wastewater	>80%	Sat	HF	Tropical (summer)	-	Yes	<i>Typha angustifolia</i>	Subsurface flow CWs may be a suitable alternative for certain pharmaceutical removal (compared to conventional WWTPs) since these HRTs are relatively short and therefore require less land area than much free water surface CWs.
Venditti et al., 2022	Lab-scale		>99.3% (lab) 95% (pilot)	uSat	Intermittent VF	Whole year	Lab cond.	yes	<i>P. australis</i> + <i>I. pseudacorus</i>	KTP is efficiently removed in unsaturated VF-CWs
Vymazal et al., 2017	Full-scale	Municipal wastewater	-31% to 92%	Sat	HF	Whole year (no winter sampling)		yes		Removal of KTP (31%) was deemed insufficient

*Sat: saturated constructed wetlands; pSat: partially-saturated constructed wetlands; uSat: unsaturated constructed wetlands;

*CWs: constructed wetlands; HF: horizontal flow CWs; VF: vertical flow CWs; FWS: free-water surface

The example of environmentally relevant substances with understudied removal and behaviour in CWs are BPS and FPN. The query on Web of Science (analogous to this for KTP) indicated that these compounds have been hardly studied (1 publication for BPS and 6 for FPN) for their removal in CWs despite their high usage and frequent detection in wastewater (Lee et al., 2015; Sadaria et al., 2017). The removal of FPN has been so far studied only in free-water surface CWs (Cryder et al., 2021; Cryder et al., 2022; Supowit et al., 2016). No data have been published for the degradation in subsurface-flow CWs. These three compounds have been selected for this study because of their relevance for household wastewater and suspected opposite degradation modes depending on the occurrence of oxic or anoxic conditions.

BPS is a plasticizer that has been used as a substitute for bisphenol A and can be released to water from plastics and other materials (such as thermal paper) (Xue and Kannan, 2019). BPS is considered an endocrine interference chemical that can be harmful to humans, animals and aquatic organisms and can be potentially transformed into more toxic substances during water processing (Zheng et al., 2018); (Wirasmita et al., 2018). The European countries have produced more than 100 kilotons of BPS used on thermal paper according to the European Chemicals Agency (ECHA, 2020). The effluents of wastewater treatment plants (WWTPs) are the main source of BPS in the environment (Lee et al., 2015). Because of its relatively low $\log K_{ow}$ BPS is not readily sorbed in WWTPs (Lee et al., 2015) and the biodegradation of BPS is frequently found to be incomplete (Campos et al., 2019). The concentration of BPS in wastewater treatment plants ranged between 27.6-31.2 ng/L (Xue and Kannan, 2019) and even up to 109 ng/L (Orona-Návar et al., 2021). The concentrations of BPS in rivers were 0.1–67 ng/L in China (L. H. Wu et al., 2018), up to 135 ng/L in Pearl River, China (Ding et al., 2020), up to 3000 ng/L in river Meuse, the Netherlands ((Ji et al., 2013), whereas its maximum concentration reached 7200 ng/L in India (Yamazaki et al., 2015). BPS was also detected in sewage sludge with a concentration of 34.5 ng/g in the USA (Yu et al., 2015). BPS was found to be non-degradable under oxic conditions, but readily degradable under anoxic conditions in various environmental matrices (Ike et al., 2006; Danzl et al., 2009). Only recently, Huang et al., (2019) reported that BPS was readily and completely biodegraded in the oxic activated sludge process and that the degradation rate was increased by the addition of humic acid. However, the fate of BPS in wastewater treatment systems remains poorly understood (Kovacic et al., 2021).

FPN is a phenylpyrazole insecticide that is used in an urban environment for structural pest control, in bait and gel products, in agriculture, topical flea and tick treatment for pets (Supowit et al., 2016). The use of spot-on products on pets and subsequent wash-off (in households or by professional groomers) has been identified as a direct pathway to WWTPs and into surface water (Sadaria et al., 2017). The concentration of FPN was detected at 12–31 ng/L in raw sewage, the effluents of WWTPs, and wetlands in the United States (Tingle et al., 2003). FPN and its transformation products are also toxic to nontarget vertebrates, including fish and gallinaceous birds, as well as to honeybees and crayfish (“Bee health_ Fipronil use to be restricted _ Agricultural and Rural Convention,” n.d.). Due to its negative effects on aquatic wildlife, the European Union restricted its use, but it still can be used on pets (Russo et al., 2019). Degradation half-lives of FPN in anoxic sediments were 4.6-18.5 days and 25.1-91.2 days in oxic sediments (Lin et al., 2008). The concentration of FPN in the surface water reached up to 5.5 µg/L (Brennan et al., 2009b). FPN was also detected in the urban catchment with the range of 1–72 ng/L in Singapore (Xu et al., 2011). In surface water, FPN varied between 1 and 22 ng/L (Montagner et al., 2019). Elevated concentrations of FPN were detected near WWTPs discharges in the range 10–500 ng/L (McMahen et al., 2016). Its concentration was detected from 204–1170 ng/L in California urban waterways (Gan et al., 2012). The concentrations of FPN-related compounds in urban and agricultural runoff were generally less than 200 ng/L from the United States Geological Survey (Supowit et al., 2016).

KTP is one of the nonsteroidal anti-inflammatory drugs (NSAID) that is toxic to microbes and other aquatic organisms (Prášková et al., 2013). KTP was shown to impair the growth and development of carp embryos and larvae and was also found to exhibit dose-dependent antagonism to human androgen receptors (Hanamoto et al., 2014). Importantly, the toxicity of KTP solution has been found to increase approximately twelve times and maintain a high toxicity level immediately after direct sunlight, indicating the presence of toxic photoproducts of KTP (Wang et al., 2018), therefore subsurface-flow CWs would be more advisable for the treatment of wastewater with considerable amounts of KTP. The maximum and median concentration of KTP in the effluent of 90 WWTPs in the EU were 1653 ng/L and 86 ng/L, respectively and the detection frequency was 48% (Loos et al., 2013). KTP was detected with a frequency of >50% in some Portuguese rivers with the highest concentration (702 ng/L) of the tested substances in the dry season (Barbosa et al., 2018). The half-life value for KTP in oxic river sediments was 5.6 d, but it increased two-fold under anoxic conditions (Koumaki et al., 2017), suggesting that KTP is preferably degraded in the presence of dissolved oxygen.

BPS and KTP, in contrast to FPN, exhibit higher biodegradability potential under oxic conditions. Both oxic and anoxic conditions can be achieved in CWs. One of the main features of CWs that govern the occurrence of these conditions is the saturation level in the system, which is associated with the type of a system: a horizontal-flow and vertical-flow system. Interestingly, saturation levels of CWs may influence the removal of organic micropollutants depending on their properties. Potentially CWs offering a mixture of conditions can be more conducive to the removal of a broad range of micropollutants. However, research focusing on CWs with mixed conditions (e.g., in partially-saturated CWs or hybrid CWs) is considered minor (Sochacki et al., 2021b). Partially-saturated CWs were reported by (Ávila et al., 2021) to offer removal efficiency higher than 90% for several antibiotics (SMX, ofloxacin, piperimidic acid, metronidazole and trimethoprim) from urban wastewater and were indicated as more efficient than unsaturated CWs. Also, superior removal of some organic micropollutants by partially-saturated CWs i.e., Caffeine (CFE), trimethoprim, and SMX was reported by (Sgroi et al., 2018). The application of both partially-saturated beds in a single CW unit was intended to enable the sequential nitrification-denitrification process (Pelissari et al., 2018, 2017).

2.2.1. Effects of plants

Plants are always presented in CWs. The presence of plants seems to favour the removal of certain pharmaceuticals and recent studies have shown that the removal of pharmaceuticals can be significantly enhanced in planted beds compared to unplanted beds (Hijosa-Valsero et al., 2010; Matamoros et al., 2007b). The dimensions of plant roots can alter the hydraulic properties of the substrate, resulting in a prolonged retention time for wastewater within the substrate (Stottmeister et al., 2003). There are direct and indirect roles of plants in CWs. The direct roles can be described as the absorption of contaminants through roots, stems and leaves in which root tissues are the main way of micropollutants removal. The function of degradation by microbial around rhizosphere zones is regarded as an indirect role (Hu et al., 2021). Micropollutants' fate in plants depends on various factors like the type of pollutants, environmental conditions, and the characteristics of the micropollutants. Wetlands plants (*I. pseudacorus*, *Typha angustifolia*, *P. australis* and *Scirpus lacustris*) can remove contaminants by harvesting because nutrients can accumulate in the plants' biomass (Gacia et al., 2019). The diffusion effect is an important process in plant uptake. Micropollutants with high polarity ($\log K_{ow} < 1$) are generally not readily taken up in significant quantities by plants (Hsu et al., 1990).

(Chen et al., 2016) and (Vymazal et al., 2017) found that hydrophobic micropollutants can be retained more than hydrophilic micropollutants in the inflow. Once their concentration reaches saturation, the absorption efficiencies of these substances will experience a sharp decline (Hu et al., 2021). Micropollutants can also be translocated to other parts of plants after being adsorbed by roots through the process of transpiration stream. Although some micropollutants can evaporate through the leaves and affect their distribution, there are higher concentrations in the above-ground parts of wetland plants. Wetland plants can degrade micropollutants and convert them into less harmful forms through processes like metabolism and mineralization (Wild et al., 2005). Although most research has been done in controlled environments, such as hydroponic studies, similar processes likely occur in natural wetlands.

The studies in Italy and India showed that CWs planted with several species (*Canna indica*, *Colocasia esculenta*, *Hymenocallis littoralis*, *P. australis*, *Arundo donax*, *Cyperus alternifolius* and *T. latifolia*) of plants have higher removal efficiencies than the CWs planted with only one species because of more abundant species of bacteria (Chen et al., 2020; Vymazal et al., 2021). There is the phenomenon of growth competition among different types of plants which can result in an unbalance in plant growth inevitably (Vymazal et al., 2021). Vegetation in CWs can store oxygen in the rhizosphere for the activities of microbial as well as resist extreme weather (Brisson and Chazarenc, 2009). It was reported that the formation of supramolecular ensembles from PPCPs and humic acids from decaying plant materials can improve the removal efficiency of PPCPs four times (Hu et al., 2021). Therefore, it is a challenge to culture a diverse ecosystem of plants in CWs.

2.2.2. Effects of substrate

There are two main categories of substrates according to their origin in CWs: natural substrates and artificial substrates. Natural substrates, like sand, soil, and gravel, offer the advantage of requiring minimal pretreatment before use. However, they come with drawbacks such as susceptibility to clogging, low adsorption capacity, and limited hydraulic conductivity. On the other hand, emerging substrates, including construction wastes and zeolite, typically possess more abundant pores (Mlih et al., 2020). This not only reduces the risk of clogging and enhances hydraulic characteristics but also facilitates the removal of organic nitrogen and phosphorus through adsorption (Wang et al., 2020). Substrates are always regarded as a platform for microbial attachment, a support system for the growth of wetland plants with

functions of adsorption and immobilization of contaminants. Its pivotal role is instrumental in the successful removal of pollutants and decreases greenhouse gases emissions (Tan et al., 2019; Xu et al., 2021). Organic substrates can enhance the process of denitrification by providing electronic donors. Moreover, this process can decrease the production of greenhouse gases (Jia et al., 2018). Moreover, emerging substrates create better aerobic conditions and more interfaces for microbial attachment, influencing the formation of biofilms and the structure of microbial communities (Tan et al., 2019). A study found that in Vertical Flow Constructed Wetlands (VFCWs) using the mixture substrates of natural substrates and artificial substrates (ceramsite/activated carbon/sand as substrate) had higher dissolved oxygen concentration than in VFCWs only with sand. This resulted in a higher abundance of aerobic bacteria in CWs with a mixture of natural substrates and artificial substrates (Fu et al., 2020). Another study demonstrated greater spatial variation in microbial community structure in zeolite CWs compared to gravel CWs (Guan et al., 2015).

3. Research questions and goals

The division of the research into three experiments allows for a detailed examination of different variable's impact on pollutant removal in CWs including water-saturated levels (saturated, partially-saturated and unsaturated), type of substrates and plants. In the first experiment, we compared the effect of three different water-saturated levels and the presence of plants on BPS, KTP and FPN removal and nitrogen removal. The results showed that CWs with partially-saturated water levels with plants performed as an all-rounder for wastewater treatment. In the second experiment, BPS and KTP were selected from the first experiment and added DCF, FCZ, SMX, SNM and ISX to the study. The impact of saturated water level, unsaturated water level and woodchips and iron chips in the substrates on BPS, DCF, FCZ, KTP, SMX, SNM and ISX was investigated. DCF, FCZ and KTP were regarded as persistent MPs to remove. All the SMX was removed in the saturated columns with woodchips and columns with iron chips. Therefore, the removal mechanism of SMX in anoxic biofilters supplemented with iron chips and woodchips was focused on. SNM and ISX are the two analogues of the SMX subunits. The removal of SMX, SNM and ISX was detected to evaluate the removal mechanism of SMX. In the third experiment, all the columns were planted because plants played a positive role in MPs removal from the results of the first experiment. The removal efficiency of clmbazole (CBZ), BPS, metoprolol (MTP), benzotriazole (BTA), 5-methyl benzotriazole (5-MeBT), acesulfame (ASF), acetaminophen (APAP), caffeine (CFE), cyclamate (CLM), DEET, ibuprofen (IBP), oxybenzone (OBZ), saccharin (SAC), sucralose (SUC), furosemide (FUR), metformin (MTF), hydrochlorothiazide (HCTZ), o-desmethyl venlafaxine (ODV), triclosan (TCS), methylparaben (MEP), gemfibrozil (GFRZ) and chloramphenicol (CAP) was studied besides DCF, FCZ, SMX, SNM and ISX. Studies about the removal efficiency of these twenty-seven MPs in CWs under different water-saturated levels with woodchips demanded were reported limitedly. In this way, outcomes of this research were expected to contribute to the optimization of CWs design and operation, leading to a new perspective on enhancing the removal of MPs from wastewater.

Here are the research questions of this thesis:

Experiment No. 1

- What is the effect of water saturation levels (unsaturated, partially saturated, saturated) and plants on the removal of FPN, BPS and KTP in CWs?

Experiment No. 2

- Can SMX degrade faster in the presence of iron chips?
- Can isoxazole and sulfonamide be used as indicators of the molecule cleavage site?

Experiment No. 3

- What is the role of woodchips in nitrogen and micropollutants removal in CWs?
- What is the role of various levels of water saturation in nitrogen and micropollutants removal in CWs?

Here are the goals of this thesis:

- (1) investigate the effect of different water-saturation levels in CWs on MPs removal from household wastewater;
- (2) evaluate the impact of the introduction of woodchips and iron chips on the MPs removal;
- (3) explore the effect of various saturation levels and the amendments on the removal of standard contaminants, in particular total nitrogen.

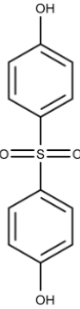
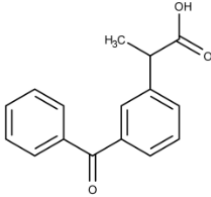
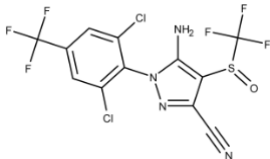
4. Materials and Methods

4.1. Experiment No.1 - removal of ketoprofen, fipronil and bisphenol S in constructed wetlands under various saturation levels

4.1.1. Reagents

The reagents used as the source of the selected organic micropollutants were BPS, KTP and FPN and all three substances were purchased from Sigma-Aldrich (Czech Republic). The reagents used in the solid phase extraction (SPE) procedure and in the LC-MS analysis were of LC-MS quality. Water used in the LC-MS analysis and the analysis of the standard wastewater parameters was ultrapure water. The reagents used for the preparation of the synthetic wastewater were represented in Table 3.

Table 3. The physicochemical properties and structures of bisphenol S, fipronil and ketoprofen,

Substance name, abbreviation and IUPAC name	CAS number	Structure	Molecular formula	Molecular weight (g/mol)	pKa	logK _{ow} logD _{ow}
Bisphenol S, BPS, <i>4-(4-hydroxyphenyl)sulfonylphenol</i>	80-09-1		C ₁₂ H ₁₀ O ₄ S	250.27	8.2 at 25 °C	1.65 (logD 2.17 at pH 7; logD -0.17 at pH 9)
Ketoprofen, KTP, <i>2-(3-benzoylphenyl)propanoic acid</i>	22071-15-4		C ₁₆ H ₁₄ O ₃	254.28	3.98	3.12 (logD 0.46 at pH 7; logD 0.09 at pH 9)
Fipronil, FPN, <i>5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile</i>	120068-37-3		C ₁₂ H ₄ Cl ₂ F ₆ N ₄ OS	437.1	Non-dissociable	4.0

Source: PubChem (<https://pubchem.ncbi.nlm.nih.gov/>); structures and the values of logD from MarvinSketch

4.1.2. Experimental set-up

An intermittently fed column system was used in this experiment to mimic the conditions occurring in full-scale CWs (Fig. 1.). The columns had a downward flow regime as the wastewater was delivered on top of the filtration bed by a pumping system. The column system was operated outdoors with a daily mean temperature range from 6.8°C to 18.6°C and was placed within an open-roofed structure to protect it from precipitation. The experimental set-up contained 12 PVC columns (diameter 20 cm, height 80 cm) grouped into 6 types based on the water saturation levels and the presence of plants, namely: unsaturated-unvegetated (uSat-uVeg); unsaturated-vegetated (uSat-Veg); partially-saturated-unvegetated (pSat-uVeg); partially-saturated-vegetated (pSat-Veg); saturated-unvegetated (Sat- uVeg), and

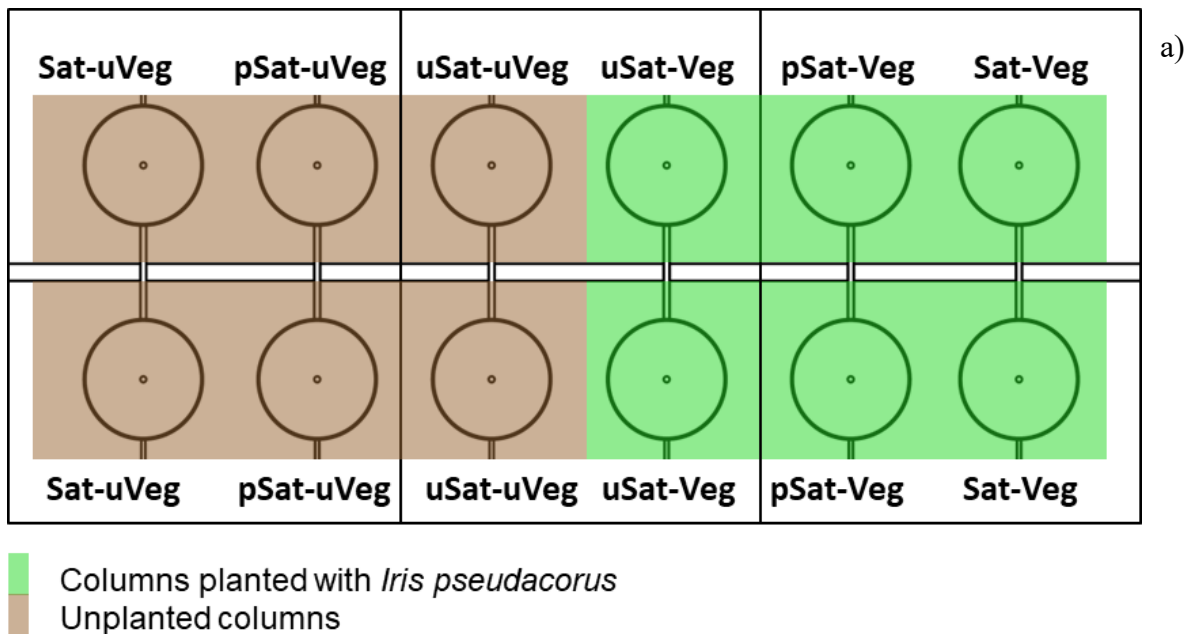


Fig. 1. Experimental setup.

saturated-vegetated (Sat-Veg) columns (Fig. 2.). The uSat-uVeg and uSat-Veg columns were operated as free-draining columns without any devices for controlling the water level. In the pSat-uVeg and pSat-Veg columns the water level was maintained at 30 cm from the bottom of

the columns, thus the upper 40 cm layer of the bed was unsaturated. The water level in the Sat-uVeg and Sat-Veg columns was at the surface of the bed media (approx. 70 cm). The Sat columns, despite the vertical flow, mimicked conditions typical of horizontal-flow CWs, which is a fully saturated bed. The columns for a specific type of system were used in duplicate. The height of the filtration media in the columns was 70 cm divided into: 5 cm bottom gravel layer (particle size 3–5 mm) and the main 65 cm layer of quartz sand (0.5–1 mm). Activated sludge mixed with synthetic wastewater was added into the columns before the experiment to inoculate the system with microorganisms. Seedlings of *Iris pseudacorus* transferred from a natural pond were planted in the Veg columns prior to the experiment. The

a)



b)

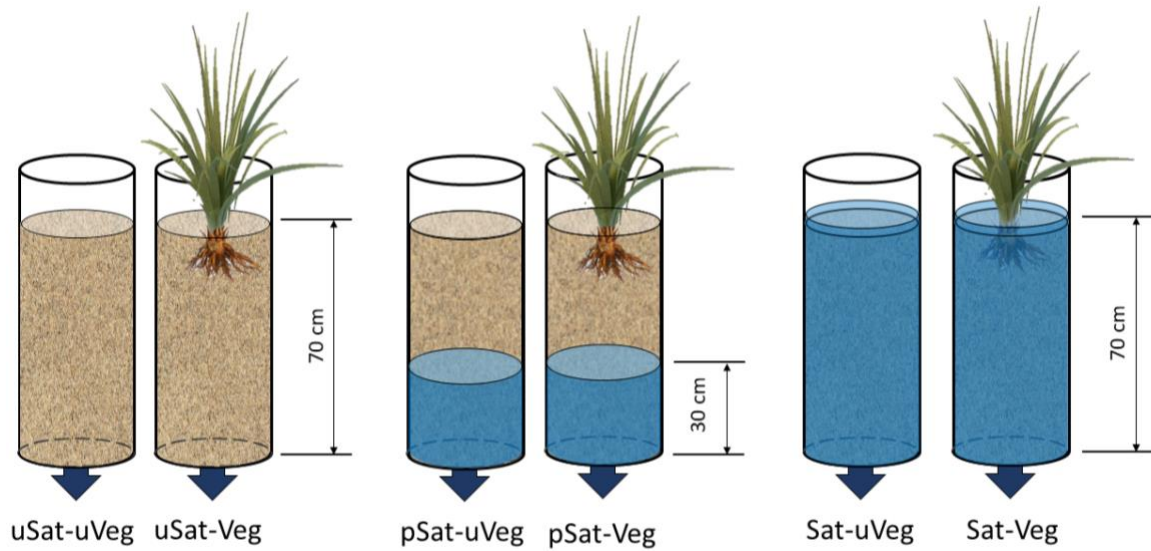


Fig 2. The experimental system: a) top-view drawing of the system with symbols of the columns and the influents used, b) types of the columns used in the experiment (not to scale).

planting density was 1 seedling per column. Each column was fed with four pulses a day at a constant dosing interval of 6 h and the volume of each pulse was 0.4 L (hydraulic loading of $0.013 \text{ m}^3/\text{m}^2$ pulse). The columns were fed using a pumping system (with an electronic controller) that distributed the influent wastewater on top of the filtering medium through a nozzle that was activated by an electro-valve. The artificial household wastewater that was used in this research was prepared according to the previously used protocol (Table 4).

Table 4. Composition of the artificial wastewater (Sochacki et al., 2021b).

Reagent	g per 100 L	Purity (application)	Manufacturer
Urea	10.4	pur.	Penta s.r.o.
NH ₄ Cl	1.6	p.a.	Penta s.r.o.
Na acetate*3H ₂ O	25.5	p.a.	Penta s.r.o.
Peptone	2.0	For microbiology	Carl Roth GmbH + Co. KG
K ₂ HPO ₄	4.1	p.a.	Penta s.r.o.
Yeast extract	13.2	For microbiology	Carl Roth GmbH + Co. KG
Skim milk	5.9	For microbiology	Sigma Aldrich
MgSO ₄ *7H ₂ O	4.1	p.a.	Penta s.r.o.
CaCl ₂ *6H ₂ O	2.8	p.a.	Penta s.r.o.
CuSO ₄ *5H ₂ O	0.001*	pur.	Penta s.r.o.
FeSO ₄ *7H ₂ O	0.045*	pur.	Penta s.r.o.
MnSO ₄ *H ₂ O	0.002*	p.a.	Penta s.r.o.
Pb(NO ₃) ₂	0.002*	p.a.	Carl Roth GmbH + Co. KG
H ₃ BO ₃	0.004*	pur.	Penta s.r.o.
Na ₂ MoO ₄ *2H ₂ O	0.002*	p.a.	Carl Roth GmbH + Co. KG
KCr(SO ₄) ₂ *12H ₂ O	0.002*	min 98 %	Carl Roth GmbH + Co. KG
ZnSO ₄ *7H ₂ O	0.003*	pur.	Penta s.r.o.
NiSO ₄ *6H ₂ O	0.002*	p.a.	Penta s.r.o.

*added from stock solution

This artificial wastewater was prepared in 80 L of tap water twice a week in an opaque barrel. FPN, BPS and KTP were added from single-compound stock solutions that were prepared in methanol. The volumes of FPN, BPS and KTP stock solutions added per 80 L of influent were the same (1 mL for each compound). The target influent concentration of FPN was 30 µg/L, and 45 µg/L BPS and KTP, each. The duration of the main part of the experiment was 102 days and lasted from mid-July to the end of October. The system was initially fed with the synthetic wastewater without FPN, BPS, KTP for 45 days to enable the adaptation of plants and microorganisms to the specific conditions. The HRT of the columns was 1.5 d, 4.5 d, 6.0 d, 8.8 d for the columns uSat-uVeg, pSat-uVeg, Sat-uVeg, Sat-Veg, respectively, which was determined in the previous experiment (Sochacki et al., 2021b).

4.1.3 Sampling and sample preparation

Wastewater sampling

The samples of wastewater were collected once a week for the analysis of FPN, BPS and KTP and for the analysis of the standard parameters (9 samplings). The samples were collected into

1L amber glass bottles for a period of 6 h between the two batches. The volume of the samples was roughly between 200-400 mL depending on the evaporation and evapotranspiration. The wastewater samples for the analysis of FPN, BPS and KTP were stored at a temperature of -25 °C. The samples were thawed at room temperature in the dark shortly before the analysis, The samples for the analysis of the standard parameters were analyzed directly on the day of sampling. For every sampling, the samples from a single batch were collected into amber glass containers placed beneath the columns.

Solid-phase extraction for fipronil, bisphenol S and ketoprofen

Purification of the water samples was carried out by SPE with Oasis Prime HLB cartridges (200 mg, 6 mL) from Waters (Milford, MA, USA) conditioned with 5 mL of methanol and 5 mL of water. After sample loading (20 ml), the cartridges were washed with 2 mL of water and further eluted with 5 mL of methanol and 5 mL of MeOH: H₂O with 0.1% formic acid. The eluates were analyzed by LC–MS/MS.

Analysis of fipronil, bisphenol S and ketoprofen

Separation was carried out using an Agilent 1290 Infinity II UHPLC system (Agilent Technologies). Chromatographic separation was achieved using a Kinetex Polar C18 analytical column 2.1x150 mm, 2.6 µm particle size from Phenomenex at a flow rate of 0.3-0.4 ml/min. The mobile phases consisted of (A) H₂O with 0.005% acetic acid and (B) acetonitrile. The gradient was 95% A at 0 min, 95% A at 0.5 min, 0% A at 6 min, 0%A at 8 min, 95% A at 8.1 min. The post time was 2 min with 95% A and the stop time 10 min. The HPLC system was coupled to an Agilent G6495A Triple Quadrupole mass spectrometer equipped with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter Acquisition software was used for data acquisition, and Agilent MassHunter Workstation software was used for data analysis. The MS parameters of all compounds were optimized and summarized in Tables 5, 6 and 7.

Table 5. QQQ conditions.

Parameter	Value
Ionization mode	+/- ESI with Agilent Jet Stream
Scan type	Dynamic MRM
Gas temperature	200 °C
Gas Flow	12 L/min
Nebulizer pressure	40 psi
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Capillary voltage	3000 V (+/-)
Nozzle voltage	0 V (+)/2000 V (-)
High-Pressure RF	110 V (+)/90 V (-)
Low-Pressure RF	70 V (+)/40 V (-)
Fragmentor	380 V

Table 6. MS/MS conditions for the compounds.

Compound Name	Precursor Ion	Product Ion	Collision Energy	Polarity
Bisphenol S	249	156	20	Negative
Bisphenol S	249	108	28	Negative
Fipronil	435	330	12	Negative
Fipronil	435	250	28	Negative
Ketoprofen	255	209	8	Positive
Ketoprofen	255	103	36	Positive
Ketoprofen	255	77	48	Positive
Ketoprofen	255	51	60	Positive

Table 7. Quantitation parameters.

Compound	Calibration range [ng/mL]	Equation	R ²	LOD [ng/mL]*	LOQ [ng/mL]**	RT [min]	Quantitation ion	m/z	Repeatability (RSD)‡	Recovery
Bisphenol S	0.1-5	y = 48.12x + 23.75	0.999	0.05	0.125	4.16	[M-H] ⁻	249	0.578%	93.7%
Fipronil	0.1-5	y = 456.74x - 30619	0.999	0.05	0.125	5.98	[M-H] ⁻	435	1.34%	93.0%
Ketoprofen	0.25-5	y = 238.43x + 211.93	0.999	0.1	0.25	5.12	[M+H] ⁺	255	5.96%	92.0%

* Limit of detection (LOD) was calculated for calibration solutions as concentration corresponding to ten times background noise

‡ Repeatability was determined from three injections from the same vial

Analysis of standard wastewater parameters

The wastewater samples before the analysis of standard parameters were filtered with 0.22 µm polyethersulfone syringe filters from Rotilabo (Carl Roth, Czech Republic). An ionic

chromatograph 883 Basic IC Plus Metrohm was used for the analysis of anions (bromides, nitrites, nitrates, sulfate and phosphate). The mobile phase used was composed of 3.2 mM sodium carbonate and 1.0 mM sodium bicarbonate. The column used for the separation of anions was Metrosep A Supp 5, 15 cm x 4 mm I.D., 5 µm particles (Metrohm 6.1006.520) and the flow rate was 0.7 mL/min and the injection volume was 20 µL. A total organic carbon/total nitrogen analyser Formacs^{HT} (Skalar) was used for the analysis of dissolved carbon (DC), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and total nitrogen (TN). The concentration of ammonium nitrogen (N-NH₄) was determined photometrically using indophenol blue method (ISO 7150-1:1984) and measuring the absorbance using the Cary 60 UV-Vis spectrometer (Agilent). pH of the wastewater was measured using WTW pH-meter 3630 IDS with an electrode IDS pH Electrode SenTix® 940.

Data analysis and statistical software used

The data normality was evaluated using Shapiro-Wilk W test. As the data indicated predominantly non-normal distribution, the Wilcoxon test to compare independent data subsets was employed using Prism Graphpad 9 software. Spearman rank order analysis was performed to determine between wastewater quality parameters in the effluents. For all the datasets, median and the median absolute deviation (MAD) were used as descriptors of the central tendency and a measure of dispersion, respectively, the statistical assessment was performed in the IBM SPSS Statistic 26 and Statistica 14 (Tibco) software.

4.2. Experiment No. 2 – Removal of bisphenol S, diclofenac, fluconazole, ketoprofen, sulfamethoxazole, sulfonamide, 5-amino-3-methylisoxazole in saturated and unsaturated biofilters amended with wood and iron chips

4.2.1. Reagents

The reagents used as the source of the selected organic micropollutants were bisphenol S (BPS) (98%), diclofenac (DCF), fluconazole (FCZ), ketoprofen (KTP), sulfamethoxazole (SMX), sulfonamide (SNM), 5-amino-3-methylisoxazole (ISX). All substances were purchased from Sigma-Aldrich (Czech Republic). The reagents used in the solid phase extraction (SPE) procedure and in the LC-MS analysis were of LC-MS quality. The water used in the LC-MS analysis and the analysis of the standard wastewater parameters was ultrapure water. The reagents used for the preparation of the synthetic wastewater are shown in Table 8.

4.2.2. Experimental setup

There were two stages in this experiment. Steel columns (diameter 3.9 cm, height 50 cm) were used in this experiment. The artificial household wastewater that was used in this research was prepared according to the previously used protocol (Table 8.). This artificial wastewater was prepared in 15 L of tap water once a week in an opaque barrel. BPS, DCF, FCZ, KTP, SMX, SNM and ISX were added from single-compound stock solutions that were prepared in methanol. The volumes of BPS, DCF, FCZ, KTP, SMX, SNM and ISX stock solutions added per 15 L of influent were the same (1 mL for each compound). The experimental system of stage 1 (Fig. 3.) consisted of 24 upflow columns. There were in total of 24 columns in stage 1: 12 unsaturated columns in the first step and 12 saturated columns in step 2. 12 unsaturated columns were all filled with sand. In the second step, four different treatments were included: Sand (Control), Sand with woodchips (10 % v/v), Sand with iron chips (10 % v/v) and Sand with woodchips and iron chips (10 % v/v each) (Table 9.). There was a total of 12 columns in stage 2 (Fig. 4.). In stage 2, a total of 12 columns were filled with sand, woodchips (10 % v/v), iron chips (10 % v/v) or a mixture of woodchips and iron chips (10 % v/v each) (Table 10.). The columns were fed using a pumping system. The target influent concentration of each organic compound was 5 mg/L. The duration of the main part of the experiment was 145 days and lasted from September to the end of January.

Table 8. Composition of the artificial wastewater.

Reagent	g per 15 L	Purity (application)	Manufacturer
Urea	1.56	pur.	Penta s.r.o.
NH ₄ Cl	0.24	p.a.	Penta s.r.o.
Na acetate*3H ₂ O	3.825	p.a.	Penta s.r.o.
Peptone	0.3	For microbiology	Carl Roth GmbH + Co. KG
K ₂ HPO ₄	0.615	p.a.	Penta s.r.o.
Yeast extract	1.98	For microbiology	Carl Roth GmbH + Co. KG
Skim milk	0.885	For microbiology	Sigma Aldrich
MgSO ₄ *7H ₂ O	0.615	p.a.	Penta s.r.o.
CaCl ₂ *6H ₂ O	0.42	p.a.	Penta s.r.o.
CuSO ₄ *5H ₂ O	0.00015*	pur.	Penta s.r.o.
FeSO ₄ *7H ₂ O	0.00675*	pur.	Penta s.r.o.
MnSO ₄ *H ₂ O	0.0003*	p.a.	Penta s.r.o.
Pb (NO ₃) ₂	0.0003*	p.a.	Carl Roth GmbH + Co. KG
H ₃ BO ₃	0.0006*	pur.	Penta s.r.o.
Na ₂ MoO ₄ *2H ₂ O	0.0003*	p.a.	Carl Roth GmbH + Co. KG
KCr(SO ₄) ₂ *12H ₂ O	0.0003*	min 98 %	Carl Roth GmbH + Co. KG
ZnSO ₄ *7H ₂ O	0.00045*	pur.	Penta s.r.o.
NiSO ₄ *6H ₂ O	0.0003*	p.a.	Penta s.r.o.

* added from stock solution



Fig. 3. Experimental biofilter columns of stage 1.

Table 9. Description of the experimental column biofilters of stage 1.

Unsaturated	S	sand (1-12)
Saturated	S	sand (13-15)
	W	woodchips (16-18) (10% v/v and sand)
	Fe	iron chips (19-21) (10% v/v and sand)
	W/Fe	iron chips (22-24) (10% v/v) and woodchips (10% v/v and sand)

Table 10. Description of the experimental column biofilters of stage 2.

Unsaturated	S	sand (1-3)
Saturated	S	sand (1-3)
	W	woodchips (4-6) (10% v/v and sand)
	Fe	iron chips (7-9) (10% v/v and sand)
	W/Fe	iron chips (10-12) (10% v/v) and woodchips (10% v/v and sand)



Fig. 4. Experimental biofilter columns of stage 2.

Influent: simulated household wastewater with SMX (number 13 influent was for columns 1,4,7,10), SNM (number 14 influent was for columns 2,5,8) and ISX (number 15 influent was for columns 3,6,9).

4.2.3. Analysis of liquid phase for organic compounds

The high-performance liquid chromatography (HPLC) coupled with a diode array detector system Ultimate 3000 (Thermo Scientific, Pragolab, Czech Republic) was used for quantification of FCZ, SMX, BPS, KTP and DCF in a mixture and the analysis of SMX, SNM and ISX in single-compound solutions. LC separations were achieved using C18 Hypersil™ Gold column (250 mm x 4.6 mm; pore size: 5 µm) (Thermo Scientific, Pragolab, Czech Republic) with a compatible precolumn (Thermo Scientific, Pragolab, Czech Republic). The mobile phase was acetonitrile (Chromasolv, for HPLC, gradient grade, >99.9%, Honeywell) and a buffer: 10 mM ammonium formate/formic acid (pH adjusted to 3.3) in ultrapure water. The separation for FCZ, SMX, BPS, KTP and DCF was obtained using a gradient mode: 0-1 min 2% acetonitrile, 33-35 min 98%, 40-45 min 2% acetonitrile. Injection volume was 20 µl for all type of samples. The compounds were quantified using linear calibration computed using at least 7 calibration levels. The retention times of the analysed compounds were: 13.57 min for FCZ, 14.14 min for SMX, 15.77 min for BPS, 16.27 min for KTP, and 24.71 min for DCF. The compounds were quantified at the following wavelengths: 260 nm for FCZ, SMX, BPS, KTP and 280 nm for DCF. The first and the second lowest calibration point was 0.05 mg/L and 0.1 mg/L, respectively. The data was evaluated by means of Dionex Chromeleon 7.2 software.

The analysis of SMX, ISX and and SNM was performed in an isocratic mode with the acetonitrile/10 mM ammonium formate/formic acid buffer ratio of 40/60, 30/70, and 30/70, respectively. The retention time was 5.64 min, 3.95 min and 3.92 min, for SMX, ISX and SNM, respectively. The compounds were quantified at the following wavelengths: 269 nm for SMX, 220 nm for ISX and 260 nm for SNM. The limit of detection values for SMX, ISX and SNM were 0.03 mg/L, 04 mg/L, and 0.02 mg/L, respectively, and the limit of quantification values were 0.08 mg/L, 0.12 mg/L, and 0.05 mg/L, respectively.

4.2.4. Analysis of solid phase by X-ray diffraction

The X-ray diffraction (XRD) analysis of lyophilized samples was performed using a desktop diffractometer Bruker D2 Phaser with an LYNXEYE XE detector (CuK α radiation, 30 kV, 10 mA, and measuring increment step of 0.022° 2 Θ , time step 2.5 s, in the range from 5° to 80° 2 Θ). The identification of all phases was performed using Diffrac. Suite EVA software (version 4.3) and the ICDD PDF-2 database (2018).

4.2.5. Transformation products

The influents and effluents of stage 2 from all the columns were analysed for the presence of transformation products of SMX. Six tentative transformation products (TPs) of SMX were analyzed by UHPLC-MS/MS. TPs analyses were performed with a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) coupled with an AB SCIEX 4000 QTRAP Hybrid Triple Quadrupole – Linear Ion Trap mass spectrometer equipped with a Turbo Ion Spray source (Applied Biosystems/MDS SCIEX, Framingham, MA, USA). The HPLC system was equipped with an UltiMate 3000 autosampler, an UltiMate 3000 RS pump, and an UltiMate 3000 thermostated column compartment. Chromatographic separation was achieved with a Kinetex F5 (Phenomenex, Torrance, CA, USA) column (100 × 2.1 mm; 1.7 μ m) at 25 °C with an injection volume of 3 μ l. The mobile phase consisted of (A) 0.1% formic acid in water and (B) acetonitrile with flow rate 0.3 ml/min. Elution was performed using the following gradient system: 0.0 min 90% A, 10% B; 3.0 min 80% A, 20% B; 7.0 min 40% A, 60% B; from 7.1 min the initial solvent composition (90% A, 10% B) was achieved with a total run-time of 10 min.

The MS/MS detector was equipped with an electrospray ionization (ESI) source. The optimum ion source parameters were as follows: temperature, 500 °C; ion spray voltage, 4000 V; curtain gas, 20 psi; ion source gas, 55 psi; ion source gas 2, 55 psi. For the identification of TPs various MS/MS modes were used. In the first step of non-targeted analysis, a screening was performed using the pseudo-multiple reaction monitoring (p-MRM) mode. The p-MRM method was built with the LightSightTM software, and a potential TPs data set was constructed based on the literature. Then, QTRAP linear ion trap scan modes, such as the enhanced mass scan (EMS) and enhanced product ion scan (EPI) modes were used. The information-dependent acquisition (IDA) mode, combining EMS with EPI, was used to maximize the information obtained in one

scan. Data recorded by the EMS-IDA-EPI method were collected in positive (ESI+) and negative (ESI-) ionization modes, while the p-MRM method data were collected only in positive ionization mode. The EMS and EPI mass ranges were from m/z 50 to m/z 700, and the scan rates were 1000 Da/s. The IDA criteria were as follows: the trigger for EPI was the 1–2 most intense ions that exceeded 100 cps; the mass tolerance was 250 mDa; former target ions were excluded for 30 s after two occurrences; the maximum rolling collision energy allowed was 80 eV in ESI+ and -80 eV in ESI-; and the dynamic background subtraction was turned on. The presence of TPs in the samples identified by the p-MRM mode was confirmed by analyzing the mass spectra recorded in the EMS-IDA-EPI mode. Non-targeted analysis was performed using a retrospective approach to mass spectral analysis.

4.2.6. Ecotoxicity assessment

Two model organisms were used for the analysis of ecotoxicity - bacterium *Aliivibrio fischeri* (ČSN EN ISO 11348-2) and seeds of *Sinapis alba*. The *Aliivibrio fischeri* bioluminescence inhibition bioassay was based on inhibition or stimulation of bacterial bioluminescence at 15 °C as a reaction to the introduced sample. To this end, 0.5 mL sample was mixed with 0.5 ml of bacterial solution and relative inhibition (Ht) a\z measured using luminometer LUMISTox 300, where “t” is 5, 15, and 30 minutes after exposure to the sample. The control sample was prepared by mixing 2% solution of NaCl and bacterial solution.

The *Sinapis alba* ecotoxicological assay a\z based on 72 h germination capacity of 30 seeds in the presence of a 10 ml sample in a Petri dish filled with filter paper. Positive values of both methods indicate the inhibition of germination or bioluminescence and negative values indicate stimulation.

4.3. Experiment No. 3 – The effect of water saturation of a vertical flow constructed wetland and woodchips on the removal of nitrogen and 27 micropollutants

4.3.1. Reagents

The reagents used as the source of the selected organic micropollutants were fluconazole (FCZ), sulfamethoxazole (SMX), diclofenac (DCF), fipronil (FPN), ketoprofen (KTP), climbazole (CBZ), bisphenol S (BPS), metoprolol (MTP), benzotriazole (BTA), 5-methyl benzotriazole (5-MeBT), acesulfame (ASF), acetaminophen (APAP), caffeine (CFE), cyclamate (CLM), DEET, ibuprofen (IBP), oxybenzone (OBZ), saccharin (SAC), sucralose (SUC), furosemide (FUR), metformin (MTF), hydrochlorothiazide (HCTZ), o-desmethyl venlafaxine (ODV), triclosan (TCS), methylparaben (MEP), gemfibrozil (GFRZ) and chloramphenicol (CAP). All substances were purchased from Sigma-Aldrich (Czech Republic). The reagents used in the solid phase extraction (SPE) procedure and in the LC-MS analysis were of LC-MS quality. The water used in the LC-MS analysis and the analysis of the standard wastewater parameters was ultrapure water. The reagents used for the preparation of the synthetic wastewater that was used in this research was prepared according to the previously used protocol (Table 11.).

Table 11. Composition of the artificial wastewater (Sochacki et al., 2021).

Reagent	g per 120 L	Purity (application)	Manufacturer
Urea	12.48	pur.	Penta s.r.o.
NH ₄ Cl	1.92	p.a.	Penta s.r.o.
Na acetate*3H ₂ O	30.6	p.a.	Penta s.r.o.
Peptone	2.4	For microbiology	Carl Roth GmbH + Co. KG
K ₂ HPO ₄	4.92	p.a.	Penta s.r.o.
Yeast extract	15.84	For microbiology	Carl Roth GmbH + Co. KG
Skim milk	7.08	For microbiology	Sigma Aldrich
MgSO ₄ *7H ₂ O	4.92	p.a.	Penta s.r.o.
CaCl ₂ *6H ₂ O	3.36	p.a.	Penta s.r.o.
CuSO ₄ *5H ₂ O	0.00012*	pur.	Penta s.r.o.
FeSO ₄ *7H ₂ O	0.054*	pur.	Penta s.r.o.
MnSO ₄ *H ₂ O	0.0024*	p.a.	Penta s.r.o.
Pb(NO ₃) ₂	0.0024*	p.a.	Carl Roth GmbH + Co. KG
H ₃ BO ₃	0.00484*	pur.	Penta s.r.o.
Na ₂ MoO ₄ *2H ₂ O	0.002*	p.a.	Carl Roth GmbH + Co. KG
KCr(SO ₄) ₂ *12H ₂ O	0.0024*	min 98 %	Carl Roth GmbH + Co. KG
ZnSO ₄ *7H ₂ O	0.0036*	pur.	Penta s.r.o.
NiSO ₄ *6H ₂ O	0.0024*	p.a.	Penta s.r.o.

*added from stock solution

4.3.2 Experimental set-up

In this experiment, an intermittently fed column system (Fig. 5.) was employed to replicate the conditions observed in full-scale CWs.



Fig. 5. The experimental set-up of the vertical-flow constructed wetlands.

The columns had a downward flow pattern, where wastewater was introduced onto the filtration bed through a pumping system. The column system was positioned outdoors and was sheltered within an open-roofed structure to shield it from rainfall. The experimental setup comprised 12 PVC columns, each with a 20 cm diameter and 80 cm height, organized into 6 categories based on water saturation levels and the types of substrates (sand or sand mixed with woodchips). These categories included partially-saturated without woodchips (pSat without woodchips), partially-saturated with woodchips (pSat with woodchips), unsaturated without woodchips (uSat without woodchips) and unsaturated with woodchips (uSat with woodchips) (Fig. 5.). The unsaturated columns were operated as free-draining columns, without any mechanisms for controlling the water level. In the partially-saturated columns, the water level was intentionally maintained at a depth of 30 cm from the bottom of the columns, leaving the upper 40 cm layer of the filtration bed in an unsaturated state. For each specific type of system, two replicates of the columns were employed. The height of the filtration media within the columns totaled 70 cm, consisting of a 5 cm layer of bottom gravel (with particle sizes ranging from 3 to 5 mm) and a primary 65 cm layer of quartz sand (with particle sizes of 0.5 to 1 mm).

Prior to commencing the experiment, seedlings of *Iris pseudacorus*, obtained from a natural pond, were planted in all the columns. The planting density was set at 2 seedlings per column. To simulate the wastewater treatment process, each column received four discrete pulses daily, distributed at a constant 6-hour dosing interval, with each pulse having a volume of 0.4 liters (resulting in a hydraulic loading rate of 0.013 m³/m² per pulse). A pumping system, controlled electronically, was used to deliver influent wastewater to the columns by means of a nozzle activated by an electro valve, ensuring uniform distribution on top of the filtering medium.

The artificial household wastewater used in this study (Table 11) was prepared following a previously established protocol (Sochacki et al., 2021b). To create this synthetic wastewater, an 80-litre opaque container filled with tap water was utilized, and this preparation process was repeated once a week. All the substances were introduced into the solution from individual stock solutions, which were originally prepared in either methanol or ultrapure water. For every 120 liters of influent, 120 µL of stock solution for compounds including FPN, KTP, CBZ, IBP, OBZ, FUR, HCTZ, ODV, TCS, GFRZ, and CAP were added. Additionally, 600 mL of stock solution for compounds such as FCZ, SMX, DCF, BPS, MTP, BTA, 5-MEBT, ASF, APAP, CFE, CLM, DEET, SAC, SUC, MTF, and MEP were added per 120 liters of influent. The target influent concentrations for the first set of compounds, including FPN, KTP, CBZ, IBP, OBZ, FUR, HCTZ, ODV, TCS, GFRZ, and CAP, were 50 µg/L each, while the target influent concentration for the second set of compounds, which included FCZ, SMX, DCF, BPS, MTP, BTA, 5-MEBT, ASF, APAP, CFE, CLM, DEET, SAC, SUC, CFE, MTF, and MEP, was 10 µg/L each. The main experimental phase spanned 85 days, starting from mid-July and concluding in mid-October. Initially, the system was supplied with synthetic wastewater without any substances for 20 days to allow the plants and microorganisms to adapt to the specific conditions.

4.3.3 Sampling and sample preparation

4.3.3.1 Wastewater sampling

Wastewater samples were collected on a weekly basis for the examination of all the substances, and standard parameters (in total, eight-time samples were collected). These samples were carefully collected in 1-liter amber glass bottles and stored for approximately 6 hours between each batch. The sample volumes typically ranged from 200 to 400 mL, depending on factors

like evaporation and evapotranspiration. The wastewater samples intended for the analysis of the substances were maintained at a constant temperature of 25°C. Just prior to analysis, these samples were thawed at room temperature in the absence of light. Samples for the analysis of standard parameters were analyzed immediately on the same day they were collected. During each sampling event, samples from a single batch were gathered and placed into amber glass containers positioned beneath the columns.

4.3.3.2 Solid-phase extraction for micropollutants

Purification of the water samples was carried out by SPE with Oasis Prime HLB cartridges (200 mg, 6 mL) from Waters (Milford, MA, USA). After sample loading (20 ml), the cartridges were washed with 2 mL of water and further eluted with 2.5 mL of methanol and 2.5 mL of MeOH: H₂O with 0.1% formic acid and 2.5 mL of MeOH: H₂O with 0.1% ammonium hydroxide. The eluates were analyzed by LC–MS/MS.

4.3.4 Analysis of micropollutants

Separation was carried out using an Agilent 1290 Infinity II UHPLC system (Agilent Technologies). Chromatographic separation was achieved using a Luna Omega PS C18 analytical column 2.1x100 mm, 3 µm particle size from Phenomenex at a flow rate of 0.3 mL/min. The mobile phases consisted of (A) H₂O with 0.5 mM NH₄F+0.01% HCOOH and (B) acetonitrile+methanol (1:1). The gradient was 98.5% A at 0 min, 0% A at 7 min to 8 min, 98.5% A at 8.1 min. The posting time was 2.9 min with 98.5% A and the stop time was 11 min. The HPLC system was coupled to an Agilent G6495A Triple Quadrupole mass spectrometer with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter Acquisition software was used for data acquisition, and Agilent MassHunter Workstation software was used for data analysis. The MS parameters of all compounds were optimized and summarized in Table 12.

Table 12. QQQ conditions.

Parameter	Value
Ionization mode	+/- ESI with Agilent Jet Stream
Scan type	Dynamic MRM
Gas temperature	150 °C
Gas Flow	12 L/min
Nebulizer pressure	35 psi
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Capillary voltage	3000 V (+) /3500 V (-)
Nozzle voltage	0 V (+) /300 V (-)
High-Pressure RF	120 V (+)/90 V (-)
Low-Pressure RF	100 V (+)/60 V (-)
Fragmentor	380 V

4.3.5. Analysis of standard wastewater parameters

Before analyzing the standard parameters, the wastewater samples were subjected to filtration using 0.22 µm polyethersulfone syringe filters obtained from Rotilabo (Carl Roth, Czech Republic). For the analysis of anions, including nitrites, nitrates, sulfate, and phosphate, an ionic chromatograph (883 Basic IC Plus Metrohm) was employed. The mobile phase was a combination of 3.2 mM sodium carbonate and 1.0 mM sodium bicarbonate. Separation of anions was carried out using a Metrosep A Supp 5 column (15 cm × 4 mm I.D., 5µm particles, Metrohm 6.1006.520) with a flow rate of 0.7 mL/min, and an injection volume of 20 µL. To analyze dissolved carbon (DC), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and total nitrogen (TN), a total organic carbon/total nitrogen analyzer (FormacsHT, Skalar) was utilized. Ammonium nitrogen (N-NH₄) concentration was determined photometrically using the indophenol blue method (ISO 7150–1:1984), and absorbance measurements were conducted with the Cary 60 UV–Vis spectrometer (Agilent). The pH and conductivity of the wastewater were measured using a WTW pH-meter 3630 IDS equipped with an IDS pH Electrode SenTix® 940.

4.3.6 Data analysis and statistical software used

Shapiro-Wilk W test was utilized for testing data normality. Given that the data exhibited primarily non-normal distributions, the Wilcoxon test was employed to compare independent subsets of data. This analysis was conducted using R Studio 3.4.1 software. Spearman rank

order analysis was carried out to examine relationships between various wastewater quality parameters within the effluents. In describing the datasets, the median and median absolute deviation (MAD) were used to indicate central tendency and measure dispersion, respectively. Statistical evaluations were performed using IBM SPSS Statistics 26 and Statistica 14 software provided by Tibco.

5. Results

5.1. Experiment No. 1

5.1.1. The removal efficiency of standard wastewater contaminants

The concentration and the removal of standard wastewater contaminants of the influent and effluents for days 45-102 are given in Table 13.

Table 13. Influent and effluent parameters (mg/L; median±MAD) and removal efficiency (in the brackets), n = 9.

Parameters	Influents	Effluents					
		uSat-uVeg	uSat-Veg	pSat-uVeg	pSat-Veg	Sat-uVeg	Sat-Veg
TN	59.7±10.0	59.7±4.9 (0.1%)	58.24±7.0 (2%)	57.70±7.1 (3%)	49.45±2.3 (17%)	32.4±14.1 (46%)	31.0±7.4 (48%)
N-NH₄	41.35±14.8 0	0.03±0.02 (99.9%)	0.02±0.01 (100%)	0.15±0.10 (99.6%)	0.21±0.20 (99.4%)	26.67±7.6 0 (35.6%)	23.60±4.9 0 (42.9%)
N-NO₂	0.006±0.00 3	0.021±0.0 09	0.018±0.0 06	0.758±0.5 11	1.275±0.8 43	0.024±0.0 24	0.027±0.0 27
N-NO₃	0.02±0.02	51.33±1.0 6	48.82±1.5 1	47.04±2.3 9	43.96±3.5 2	0.03±0.02	0.36±0.23
TOC	61.9±19.9	6.7±0.3 (89%)	7.1±0.3 (88%)	9.1±1.7 (85%)	9.8±1.3 (84%)	17.5±1.5 (72%)	16.5±2.1 (73%)
IC	77.6±17.6	10.3±2.8 (87%)	13.9±2.1 (82%)	14.7±2.0 (81%)	16.5±7.3 (79%)	87.3±7.3 (-12%)	92.2±8.7 (-19%)
pH	8.6±0.1	7.8±0.1	7.9±0.1	8.5±0.7	9.3±0.9	8.4±0.3	8.2±0.2
Sulfate	41.1±4.3	48.5±2.8 (-18%)	48.9±2.4 (-19%)	45.8±4.8 (-11%)	46.2±3.5 (-12%)	6.4±6.3 (84%)	21.2±5.5 (48%)
Phosphate	30.2±4.2	16.2±3.6 (46%)	14.0±2.7 (54%)	6.3±4.3 (79%)	5.0±4.7 (83%)	15.3±13.3 (49%)	3.2±1.8 (89%)

The removal of TN was negligible in the uSat columns. Within the group of the pSat columns only the vegetated columns provided significant yet low removal of 17%. This suggests that plants are an important factor in the removal of TN in partially-saturated CWs. This enhanced removal of TN can be due to plant uptake or stimulation of denitrification by the root exudates containing organic carbon. The activity of denitrifying bacteria can be indirectly proven by increased pH values in the effluents of the pSat columns and increased concentrations of N-NO₂, which could have resulted from partial denitrification. Denitrifying bacteria were found in all layers of full-scale partially-saturated vertical flow CWs (Pelissari et al., 2017), but the denitrifying gene expression was enhanced mainly in the bottom layer of a partially-saturated CW (Pelissari et al., 2018). The removal of TN in the pSat columns could be probably improved

by providing a sufficient amount of bioavailable organic carbon for denitrifying microorganisms. This could be, for example, achieved by using woodchips as an amendment of the filtering medium or by adjusting the depth of the saturated layer. The Sat columns provided moderate (approx. 50%), however, the highest removal of TN. The effect of plants on the removal of TN in the Sat columns was insignificant ($p \geq 0.05$).

N-NH₄ was almost completely removed in the uSat and pSat with an insignificant ($p < 0.05$) effect of the presence of plants. The pSat columns provided slightly lower removal (by 0.3%-0.6%). The removal of N-NH₄ in the Sat type of columns was moderate and considerably lower than in the other types of columns.

The removal of TOC was the highest in the uSat and the removal was slightly lower in the pSat columns. The lowest removal of TOC was observed in the Sat columns. The effect of saturation on the removal of TOC was statistically significant ($p < 0.05$), but the plants played a significant role in the removal of TOC only in the uSat columns ($p < 0.05$), however, the differences were negligible from the process point of view.

There was a significant ($p < 0.05$) effect of plants on the removal of phosphate in the uSat and Sat columns. The plants enhanced the removal of phosphate with the most pronounced effect in the Sat columns. The removal of phosphate was the highest in the vegetated pSat and Sat columns, without a significant effect of the saturation. Interestingly, pSat-uVeg columns provided the highest removal of phosphate when compared with the uSat and Sat counterparts.

The removal of sulfate in Sat-uVeg and Sat-Veg columns was 84% and 48%, respectively, with significant ($p < 0.05$) effect of plants that decreased the removal efficiency. This effect of plants can be probably attributed to oxygen transfer to the root zone, which could have inhibited sulfate-reducing bacteria. Sulfate was released from the other types of columns suggesting dissolution of sulfur-bearing phases (Harvey and McCormick, 2009). Interestingly, partially-saturated columns were not able to sustain sulfate reduction, which could have been due to the lack of organic carbon (depleted in the upper part of the columns) or due to increased concentrations of competing electron acceptors. The quick depletion of TOC in the uppermost 20-cm layer of vertical-flow CWs has been reported in the literature (Olsson et al., 2011; Boog et al., 2014). The removal of sulfate by partially-saturated columns in this study is

comparatively lower than 42% removal reported by Saeed and Sun (2017), for partially-saturated vertical flow system planted by *Canna indica*.

The pH value was lower in the effluents of the uSat columns when compared with the influent values. This can be attributed to the effect of the nitrification process. For the pSat and Sat columns the pH columns, the pH in the effluents was only slightly lower than the influent value, because of the co-occurrence of nitrification and denitrification and sulfate reduction (only in the Sat columns) processes. The exception was the pSat-Veg columns, in which the effluent pH was considerably higher than in the influent. This was probably due to the denitrification process and the lack of organic acids that could have been produced in the Sat columns under more reducing conditions.

The removal of IC was positive in uSat and pSat columns suggesting the effect of the nitrification process and insignificant effect of alkalinity increasing processes like denitrification and sulfate reduction. On the other hand, a moderate increase in IC occurred in the Sat and columns suggesting the effect of denitrification and sulfate reduction processes.

5.1.2. The removal of organic micropollutants in the constructed wetlands

5.1.2.1. Overall results

The influent and effluent concentrations of the studied micropollutants and their removal efficiency are shown in Fig. 6.

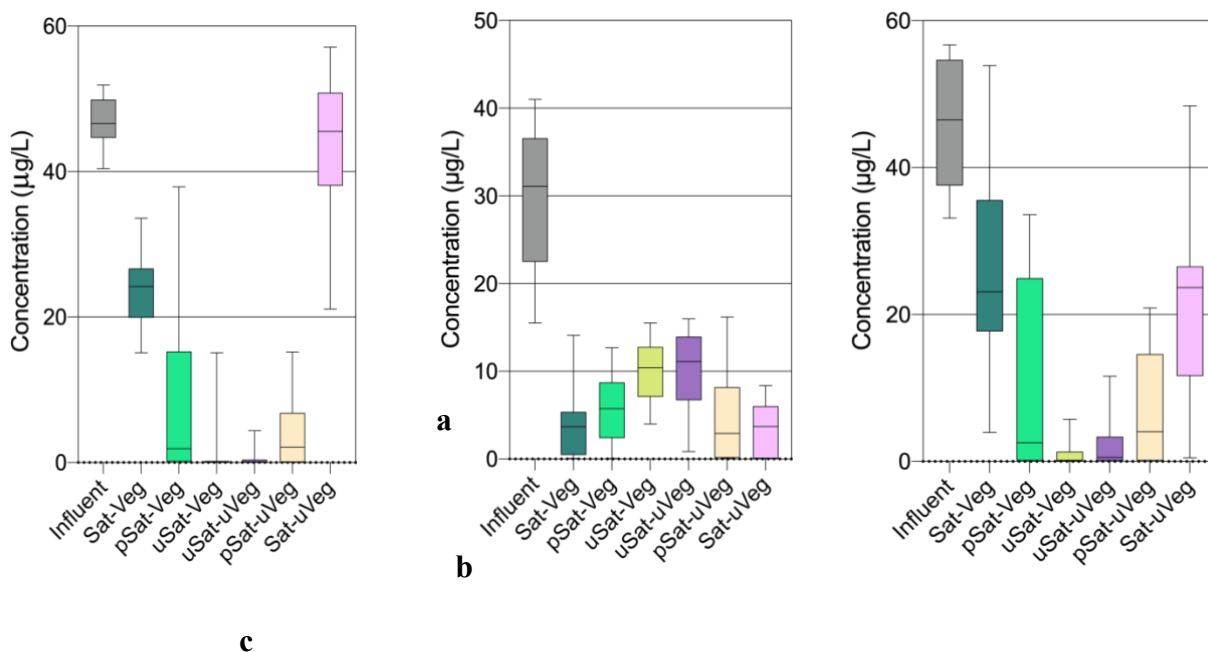


Fig. 6. The concentration (median on bars and MAD as error bars) of the organic micropollutants in the influents and effluents of the experimental columns: a) bisphenol S, b) ketoprofen, c) fipronil; data for days 45-102, n=9.

Unsaturated columns provided the highest and nearly complete BPS removal as compared to the columns with other saturation levels. Significant differences in the removal efficiency among counterpart ($p < 0.05$) (Table 13) columns with saturated, partially-saturated and unsaturated beds were observed. Interestingly, partially-saturated columns were also efficient for the BPS removal, but they offered slightly lower efficiency (by approx. 4%). The presence of plants exhibited a significant effect on the removal of BPS only in the case of the Sat columns (Table 14). Namely, the presence of plants increased the removal by 46%, from negligible in the Sat-uVeg columns to almost 50% in the Sat-Veg columns.

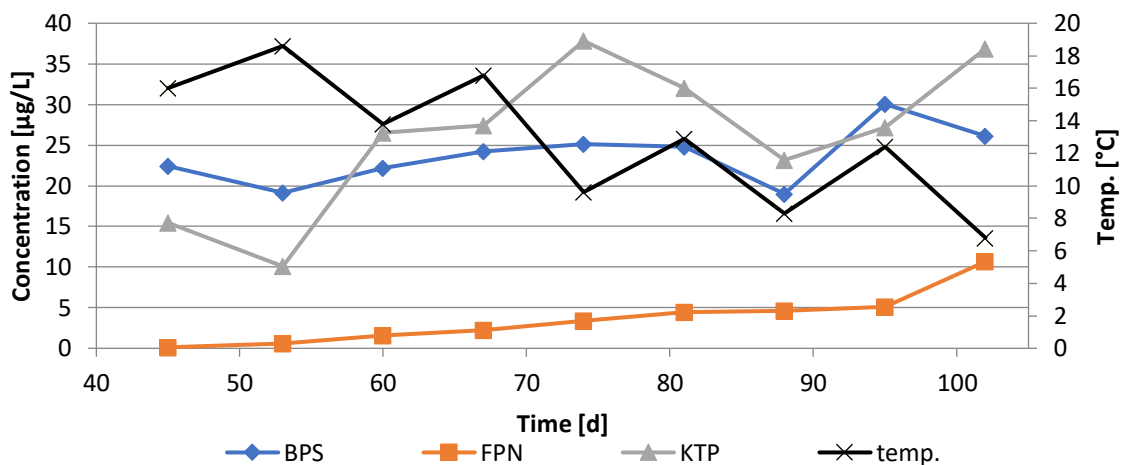
The uSat columns provided also the highest removal of KTP, which was 98.8% and 99.7%, in the uVeg and Veg columns, respectively. The removal in the pSat and Sat counterparts was lower by 5-7% and approx. 50%, respectively, and the differences were statistically significant. The plants had a significant effect on the removal of KTP only in the uSat columns and they improved the removal by 1%.

In contrast to BPS and KTP, the highest removal efficiency for FPN was observed in the pSat-uVeg, Sat-uVeg and Sat-Veg columns and was approx. 90% without significant differences

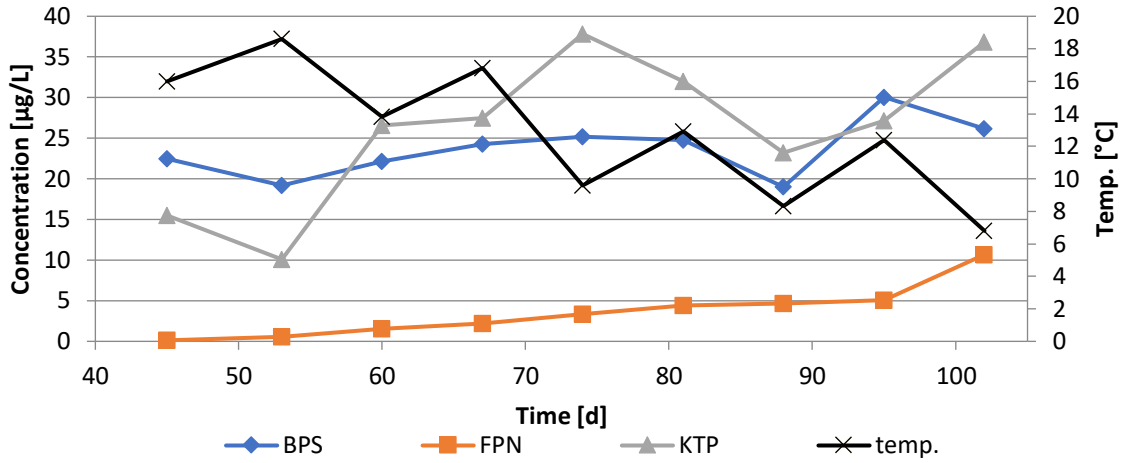
between the three types of the columns. Interestingly, the uSat columns provided considerably lower removal efficiency for FPN (approx. by 25%). The effect of the presence of plants was observed to be significant only in the pSat columns, in which the presence plants with the removal of FPN lower by 9.1%.

5.1.2.2. Temporal changes in the concentration of micropollutants

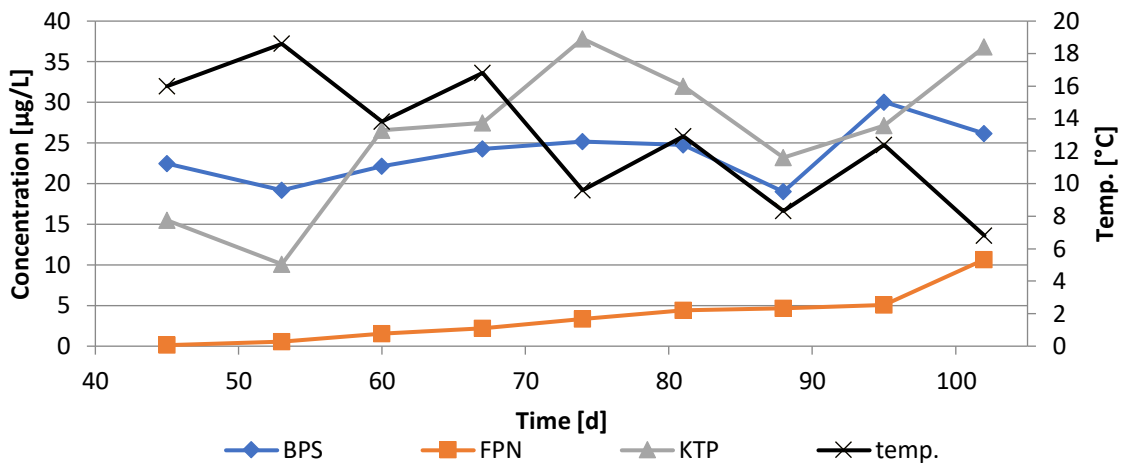
The concentration of BPS in the effluents of Sat-Veg and uSat-Veg columns was stable until day 88 or slightly increasing. In the case of columns pSat-Veg, BPS concentration was gradually increasing from days 53 up to the last day of the experiment, reaching almost 5- fold concentration increment (Fig. 7). The concentration of BPS in the effluent of the Sat-uVeg columns was higher than in the effluent from day 67 until the end of the experiment. This might be due to the deconjugation process occurring in saturated columns as conjugated metabolites are reverted to the parent compounds (Buarque et al., 2019; Styszko et al., 2021b) or the desorption from the filtering material. The sorption and desorption process of BPS in CWs with the effect of temperature should be a matter of future research. In terms of the presence of plants, it can be noticed that the plants indicated minor contribution for the BPS removal in unsaturated conditions as both planted and unplanted treatments were not showing significant difference ($p>0.05$).



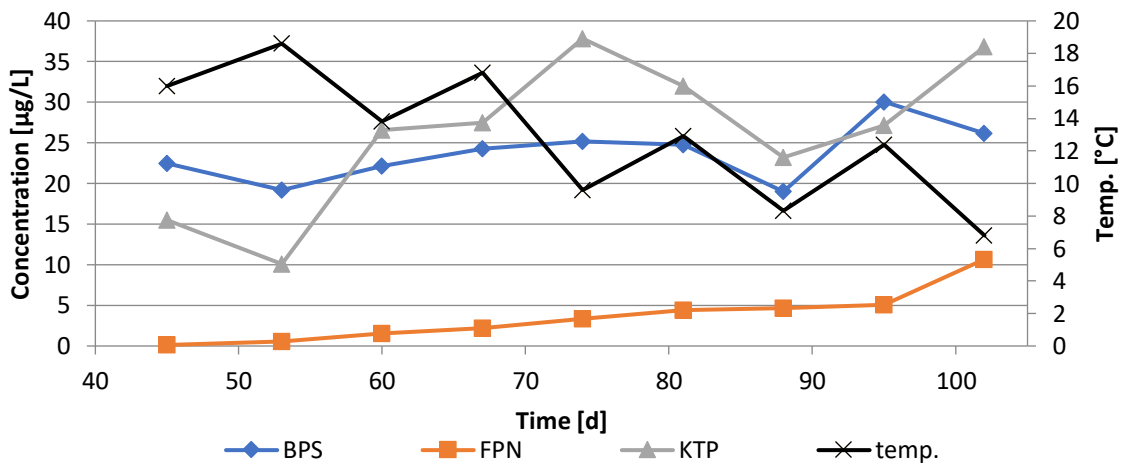
Sat-Veg



Sat-uVeg



pSat-Veg



pSat-uVeg

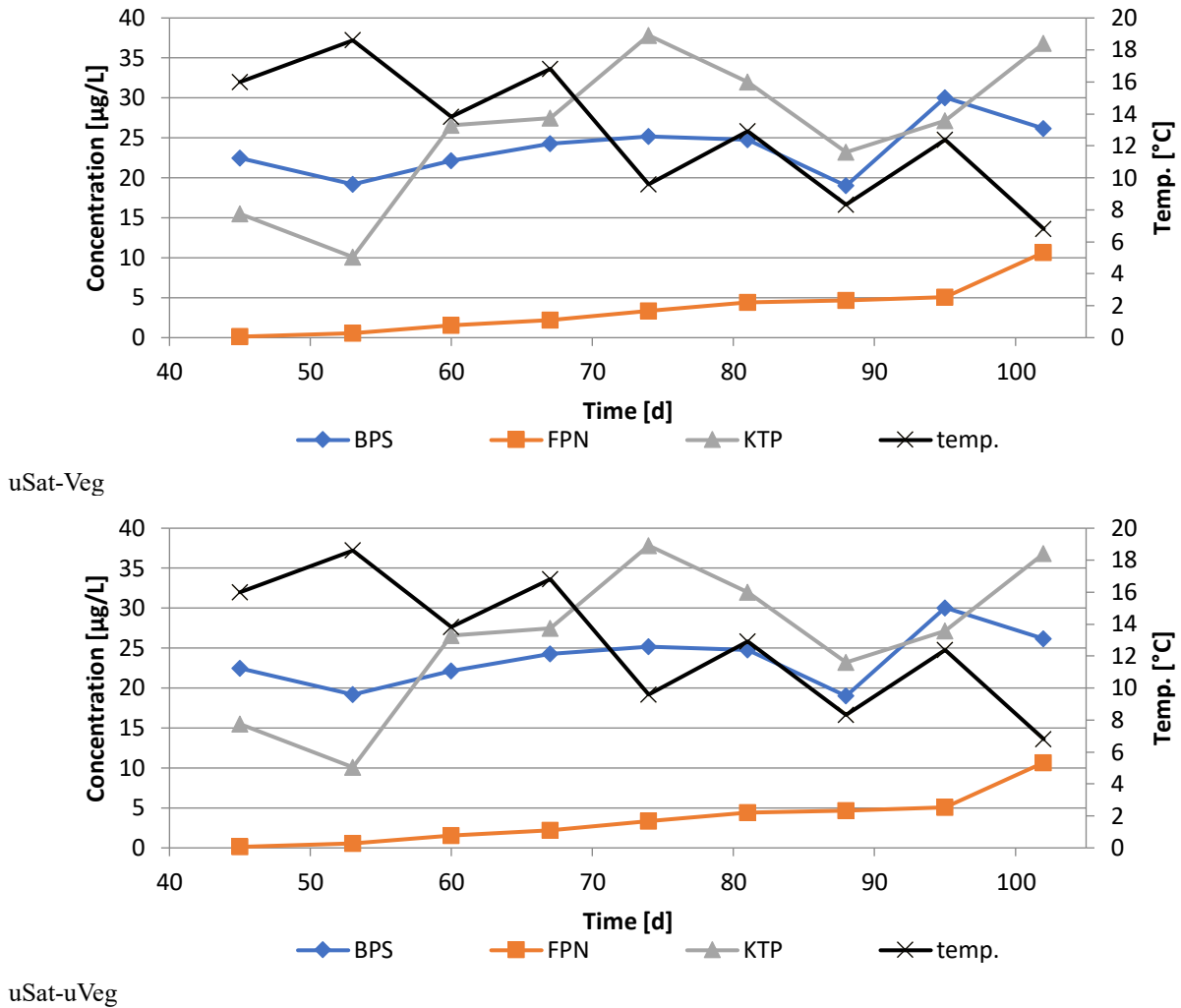


Fig. 7. Time-dependent concentrations of bisphenol S (BPS), ketoprofen (KTP) and fipronil (FPN) in the effluents.

The concentration of KTP in unsaturated columns was gradually decreasing after the period of 60 days up to the last day of operation (SM 10). The KTP effluent concentrations for the pSat-Veg and Sat-Veg columns had a relatively stable profile after day 67 after initial increasing trend. For the uSat-Veg columns, the KTP effluent concentrations decreased from initial peak values to stabilize after day 67. The behavior of KTP was analogous in the unplanted columns.

The concentrations of FPN in the effluents of the saturated columns were visibly increasing between days 53 and 74 and afterwards a pseudo-steady state was reached. The temporal behavior of FPN in the pSat-Veg columns followed a similar pattern, but in the pSat-uVeg a steady increase until the end of the experiment was observed with a transitory plateau period

between days 88-95. This latter observation was also valid for the uSat columns, but with a slight shift of the plateau period to days 81-88. Importantly, KTP and FTP exhibited a similar concentration pattern in the effluents of the pSat columns. This suggests that the removal of FPN was not governed by adsorption. Otherwise, considerably greater retardation of this compound would have occurred in these conditions compared to much more mobile compound KTP (based on $\log D_{ow}$ values). The temporal characteristics of the removal of FPN are not strongly dependent on the HRT, because the concentration profile is comparable for all the types of columns (Fig. 8).

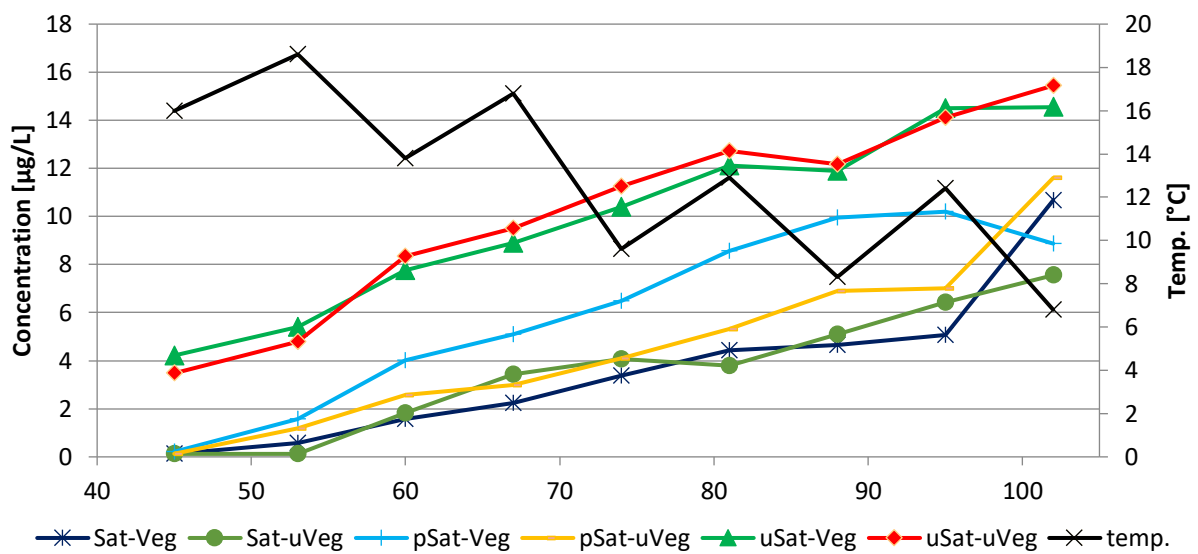


Fig. 8. Concentration profile of fipronil in the effluents (Sat-Veg-saturated columns with plants, Sat-uVeg-saturated columns without plants, pSat-Veg-partially saturated columns with plants, pSat-uVeg-partially saturated columns without plants, uSat-Veg-unsaturated columns with plants, uSat-uVeg-unsaturated columns with plants, temp-temperature).

5.2. Experiment No. 2

5.2.1. Results – removal of five micropollutants

The investigation into the removal of BPS, KTP, DCF, SMX, and FCZ in various saturated systems yielded distinct outcomes. BPS and KTP exhibited high removal efficiency in unsaturated biofilters. BPS demonstrated comparable removal efficiency in saturated biofilters. The highest removal efficiency of BPS was in Sat-WCh (79%), while unexpectedly exhibiting a considerably lower removal rate in Sat-Fe (16%), indicating the limited effect of iron chips in BPS removal under saturated columns. KTP, on the other hand, displays diverse removal efficiencies, reaching its peak at 84% in unSat-all, but registering negative removal efficiency in Sat-CTRL and Sat-Fe-Wch conditions. However, the removal of DCF, SMX, and FCZ was negligible in unsaturated columns. FCZ and DCF were found to be recalcitrant compound, but the present experiment that an anoxic process has higher potential for the removal of this compound. For DCF, it was only removed in saturated columns, indicating the anoxic condition displayed the positive impact of the filtration processes but with a modest removal efficiency which is similar to the removal trend of FCZ. In contrast, SMX and BPS exhibit more pronounced variations in removal efficiency. SMX was found recalcitrant in the unsaturated columns, but it was completely removed in the columns Sat-Fe and Sat-Fe-Wch columns, suggesting the effectiveness of these saturated conditions. The results are presented in Table 14.

Table 14. The removal efficiency of fluconazole, sulfamethoxazole, bisphenol S, ketoprofen and diclofenac.

	unSat-all	Sat-CTRL	Sat-WCh	Sat-Fe	Sat-Fe-Wch
Fluconazole	-4%	-5%	16%	9%	20%
Sulfamethoxazole	3%	2%	11%	100%	100%
Bisphenol S	54%	63%	79%	16%	69%
Ketoprofen	84%	-254%	29%	21%	-354%
Diclofenac	-7%	11%	22%	11%	25%

* Sat-CTRL-saturated columns with sand, Sat-WCh-saturated columns with sand and woodchips, Sat-Fe-saturated columns with sand and iron chips, Sat-Fe-WCh-saturated columns with sand, woodchips and iron chips.

5.2.2. Removal of sulfamethoxazole

SMX was removed more than 99% in the columns with iron chips including Sat-Fe columns and Sat-Fe-Wch columns which indicate iron chips have a comparable effect on SMX under anoxic conditions. The results are presented in Fig. 9. There was a modest removal efficiency

of 11% in the Sat-Wch columns. SMX was removed negligibly in the controlled columns filled with sand in which the removal efficiency was 3%.

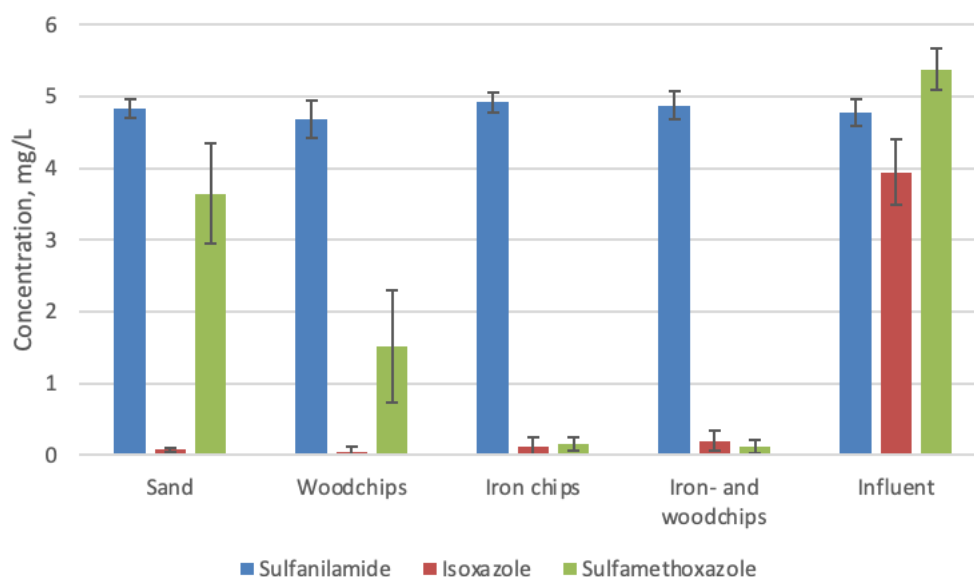


Fig. 9. Concentration of sulfamethoxazole, Sulfonamide and 5-amino-3-methylisoxazole in the influent and effluent.

5.2.3. Ecotoxicity assessment

The luminescence inhibition of SMX in the influents was more than 50%. The observed luminescence inhibition values due to the exposure to the influent and effluents on the 16th December 2022 and 2nd February 2023 are given in Fig. 10. The luminescence inhibition of the effluents from the columns with iron chips and columns with iron chips and woodchips decreased significantly compared to the influents. A slight decrease of luminescence inhibition efficiency was observed in the controlled columns (Sand) and columns with woodchips had a modest luminescence inhibition efficiency following. Additionally, there was negative luminescence inhibition (luminescence stimulation) in the columns with iron chips and columns with woodchips which was the same as the trend of the luminescence inhibition of SNM. The luminescence inhibition of ISX was negative in all influents and effluents.

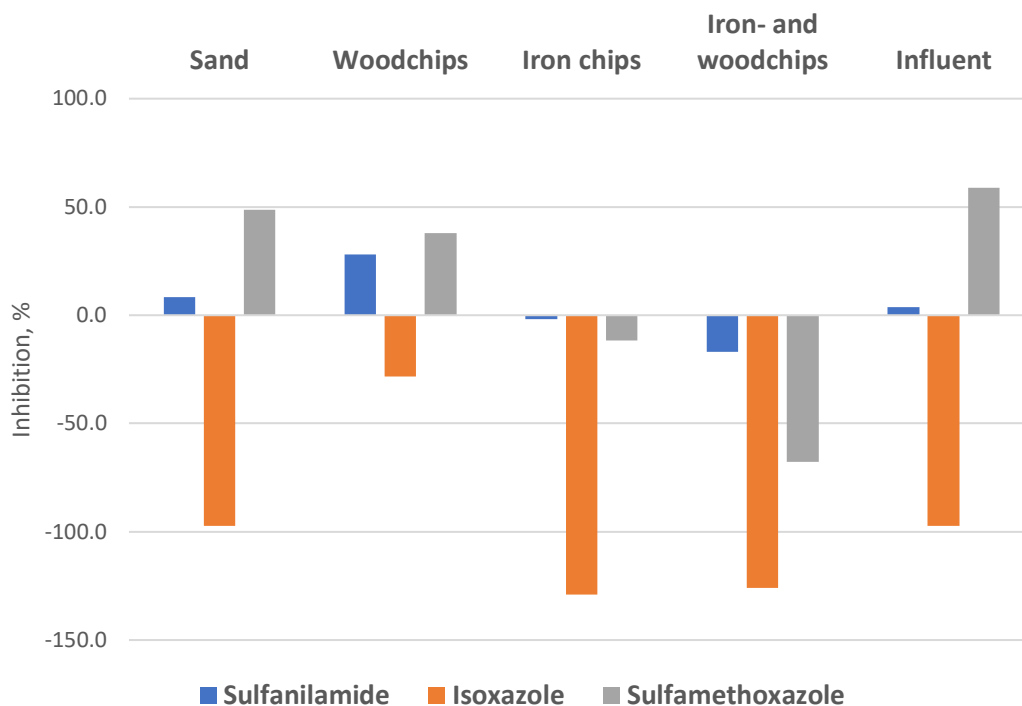


Fig. 10. Influent and effluent ecotoxicity determined by the *Sinapis alba* bioassay.

The luminescence inhibition of ISX in the influents was above 40% followed by the effluents from the columns with iron chips and woodchips with above 20% luminescence inhibition. The results are shown in Fig. 10. The luminescence inhibition of the effluents from the columns with woodchips and columns with iron chips decreased significantly compared to the influents with the luminescence inhibition below 10%. Additionally, the luminescence inhibition decreased over 50% of the influents. The samples for ecotoxicity were taken on 8th Decmenber 2022. The correlation between the effluent and influent concentrations and ecotoxicity was shown in Table 15. It can be seen, that the correlation between SMX and *Aliivibrio fischeri* is weak, suggesting that the SMX itself might not be toxic, but the transformation can be more toxic than the parent compound.

Table 15. The correlation between the effluent and influent concentrations and ecotoxicity.

	<i>Sinapis alba</i>	<i>Aliivibrio fischeri</i>	Sulfonamides	5-amino-3-methylisoxazole	Sulfamethoxazole
<i>Sinapis alba</i>	1.0000	-0.6882	0.3491	-0.3528	0.5901
<i>Aliivibrio fischeri</i>	-0.6882	1.0000	-0.4630	0.7134	-0.3344
Sulfonamides	0.3491	-0.4630	1.0000	-0.2057	-0.3403
5-amino-3-methylisoxazole	-0.3528	0.7134	-0.2057	1.0000	-0.1404
sulfamethoxazole	0.5901	-0.3344	-0.3403	-0.1404	1.0000

The luminescence inhibition of the columns with iron chips and woodchips was a little higher than the controlled columns. For SFM, negative luminescence inhibition (luminescence stimulation) was found in the columns except for columns with iron chips and woodchips. Negative luminescence inhibition of SMX occurred in the influents, columns with sand and columns with iron chips and woodchips (Fig. 11).

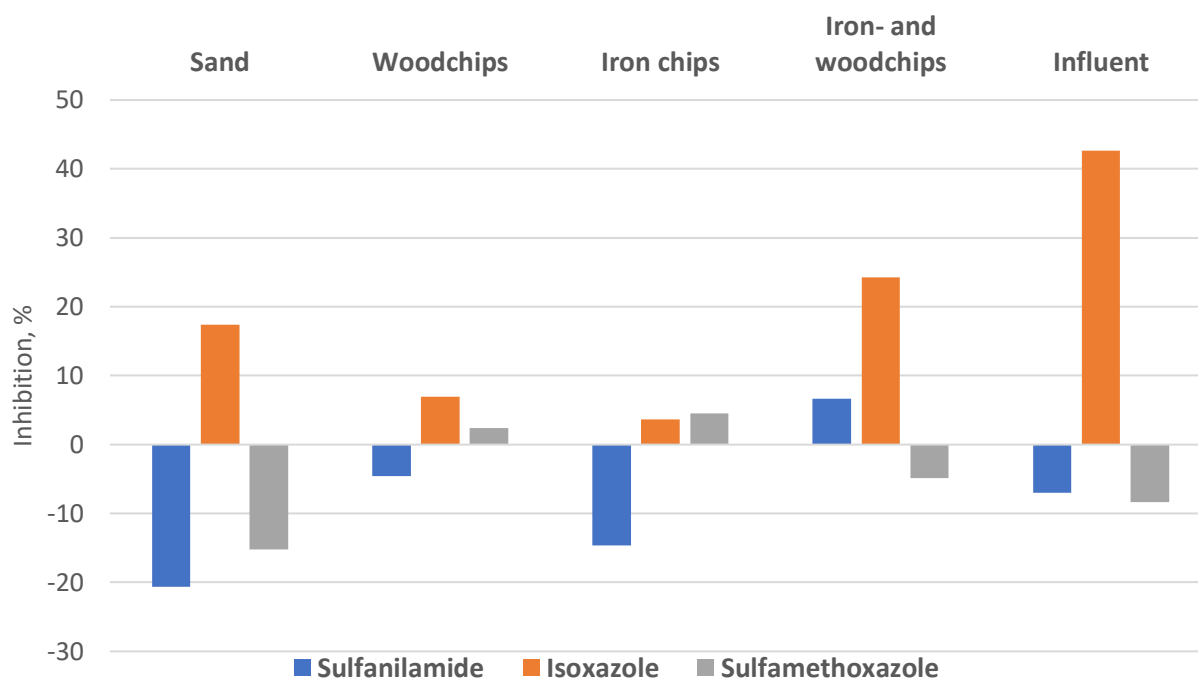


Fig. 11. Influent and effluent ecotoxicity determined by the Microtox bioassay (*Aliivibrio fischeri*).

5.2.4. Transformation products

There is the tentative information and structures of six kinds of transformation products of SMX (Table 16. and Table 17.). TP1 transformation: 3-amino-5-methylisoxazole ring cleavage;

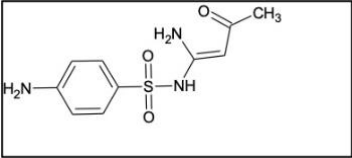
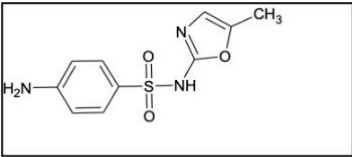
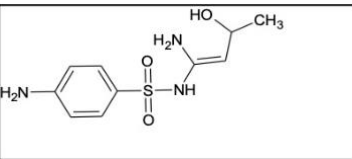
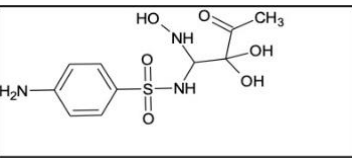
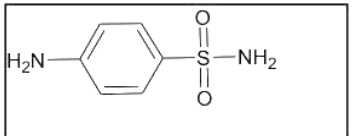
TP2 transformation: isomerization of the 3-amino-5-methylisoxazole ring; TP3 transformation: 3-amino-5-methylisoxazole ring cleavage; TP4 transformation: 3-amino-5-methylisoxazole ring cleavage; TP5 unidentified; TP6 transformation: 3-amino-5-methylisoxazole ring cleavage, formation of SNM.

Table 16. Summary of tentatively identified transformation products of sulfamethoxazole.

	SMX	TP1	TP2	TP3	TP4	TP5	TP6
Retention time (min)	6.37	1.62	2.32	1.33	5.13	8.33	1.25
Transition ions/ product mass, m/z [M+H] ⁺	254	256	253.9	258	307	279	173.9
Fragmentation ions	156; 108; 92; 147; 188	156,9; 108; 101; 214	156; 140; 108; 92	156; 108; 92; 214; 85	238; 220; 289; 169	201; 149	156; 92; 108; 76
Influent		nd.	nd.	nd.	nd.	nd.	nd.
Sand		2.00E+05	7.00E+04	6.00E+04	nd.	nd.	nd.
Woodchips		6.00E+05	2.00E+05	1.50E+04	3.00E+03	5.00 E+03	nd.
Iron chips		1.20E+06	5.00E+04	4.00E+04	4.00E+03	4.00 E+03	nd.
Iron chips and woodchips		6.60E+05	5.00E+04	4.00E+04	4.00E+03	4.00 E+03	4.00E+0 4

*TP-transformation products, SMX- sulfamethoxazole.

Table 17. The structures of transformation products of sulfamethoxazole.

Name	Structure	Molecular formula
TP1		C ₁₀ H ₁₃ O ₃ N ₃ O ₃ S
TP2		C ₁₀ H ₁₁ N ₃ O ₃ S
TP3		C ₁₀ H ₁₄ N ₃ O ₃ S
TP4		C ₁₀ H ₁₄ N ₃ O ₆ S
TP5	Unknown	Unknown
TP6		C ₆ H ₈ N ₂ O ₂ S

*TP-transformation products

There is no noticeable difference in the mode of transformation since the cleavage of the 3-amino-5-methylisoxazole ring cleavage was confirmed in all the types of columns. The superiority of iron chips in the removal of SMX may lie in greater reaction kinetics, which is indirectly and only partially indicated by the presence of SNM (TP6) as the transformation of SMX. Based on the negligible removal of SNM, it can be assumed that this compounds as a TP of SMX might not undergo further degradation posing an environmental risk.

5.3. Experiment No. 3

5.3.1. The removal efficiency of standard wastewater contaminants

The concentration and the removal of standard wastewater contaminants of the influent and effluents for days 21–85 is given in Table 18.

Table 18. Influent and effluent parameters (mg/L; median \pm MAD) and removal efficiency (in the brackets), n = 8 (MAD – median average deviation).

	Influents	Effluents			
		pSat	pSat-Woodchips	uSat	uSat-Woodchips
TN	50.4 \pm 0.8	46.7 \pm 2.1 (7%) ^	27.0 \pm 6.9 (46%) ^	48.5 \pm 1.6 (4%) ^	44.0 \pm 2.4 (15%) ^
N-NH ₄	6.9 \pm 1.6	12.2 \pm 5.4 (-77.1%)	0.4 \pm 0.3 (94.3%) ^	0.1 (99.1%) ^	0.1 \pm 0.1 (98.1%) ^
N-NO ₂	0.58 \pm 0.10	15.48 \pm 11.00	9.25 \pm 4.49	0.06 \pm 0.05	0.15 \pm 0.22
N-NO ₃	24.66 \pm 1.34	140.56 \pm 31.54	92.76 \pm 22.46	191.59 \pm 5.66	170.14 \pm 16.55
TC	160.5 \pm 8.2	47.1 \pm 16.0 (71%)	65.7 \pm 8.5 (59%)	21.6 \pm 2.0 (87%)	60.1 \pm 11.0 (63%)
TOC	115.8 \pm 8.6	7.6 \pm 1.0 (93.4%) ^	11.3 \pm 1.4 (90.2%) ^	5.4 \pm 0.5 (95.3%) ^	11.9 \pm 1.4 (89.7%) ^
IC	45.4 \pm 1.8	39.4 \pm 15.9 (13%)	54.6 \pm 6.1 (-20%)	16.7 \pm 2.2 (63%)	48.5 \pm 9.5 (-7%)
Sulfate	51.8 \pm 7.3	59.2 \pm 1.4 (-14%)	60.2 \pm 2.7 (-16%)	57.7 \pm 2.3 (-11%)	56.3 \pm 2.3 (-9%)
Phosphate	15.4 \pm 0.2	18.5 \pm 3.9 (-20%)	14.2 \pm 3.9 (8%)	20.1 \pm 2.2 (-30%)	9.0 \pm 8.6 (42%)
Conductivity	838 \pm 14.5	1010 \pm 17.5	947 \pm 25.5	951 \pm 13	1014 \pm 23
pH	8.0 \pm 0.2	7.6 \pm 0.3	8.1 \pm 0.5	7.9 \pm 0.1	7.1 \pm 0.4

^ Difference with respective influent at p < 0.05.

*pSat-partially saturated columns, uSat-unsaturated columns.

Total Nitrogen

The removal of TN was negligible in the pSat columns and uSat columns without woodchips. In the partially-saturated columns with woodchips, there is the highest removal efficiency of 46%. The nitrite concentration and pH values in the effluent of pSat columns with woodchips were positively related to TN removal, which indicates partial denitrification. Woodchips in the columns have a significant function in increasing TN removal (p < 0.05) which may be because woodchips as the bioavailable carbon source can enhance the activities of denitrification microorganisms. This amendment of woodchips is regarded as an improvement of our previous study (Ren et al., 2023) in which the highest TN removal efficiency was only

17% in pSat columns. The effect of woodchips on the removal of TN in the pSat columns and uSat was significant ($p < 0.05$).

Ammonium nitrogen (N-NH₄)

N-NH₄ was almost completely removed in the pSat columns with woodchips and uSat columns with a significant difference ($p < 0.05$). The concentration of N-NH₄ in pSat columns without woodchips was increasing with a big amount. The removal efficiency of N-NH₄ concentration in pSat and uSat is significant in the presence of woodchips ($p < 0.05$).

Total Organic Carbon

In the partially-saturated columns, the highest removal efficiency occurred in uSat columns due to the aerobic environment which is beneficial to the degradation of organic carbon. The removal of TOC was the highest in the uSat without woodchips and the removal was slightly lower in the pSat columns without woodchips. The lowest removal of TOC was observed in the uSat columns with woodchips. The addition of woodchips resulted in the TOC concentration in the columns with woodchips being always higher than in other columns. The effect of woodchips on the removal of TOC was statistically significant ($p < 0.05$). Without woodchips, the removal efficiency of uSat columns surpassed pSat ($p \geq 0.05$), however, the differences were negligible from the process point of view. When there were woodchips present, there was no significant difference between the columns which indicates that woodchips could affect the TOC removal more than water-saturated levels.

Phosphate (PO₄³⁻)

There was a significant ($p < 0.05$) effect of woodchips on the removal of phosphate in the uSat and pSat columns. The woodchips enhanced the removal of phosphate in all the columns. The removal of phosphate was the highest in the Sat columns with woodchips which was much higher than in pSat columns with woodchips. Interestingly, the concentration of phosphate in the columns without woodchips all increased.

Sulfate (SO₄²⁻)

The concentration of sulfate was almost increased in all effluent. The results may be due to the amount of oxygen accumulated in pSat columns and uSat columns without woodchips. The highest sulfate concentration was in the effluents of the pSat columns. An aerobic environment

is not beneficial to sulfate-reducing bacteria growth and results in the inhabitation of sulfate removal. Woodchips as an organic carbon source in the columns increased the concentration of sulfate in the effluents.

pH

The pH in the effluent of the uSat columns and the pSat columns without woodchips were lower than the influent because abundant oxygen causes nitrification to proceed adequately while denitrification was limited. The exception was the pSat columns with woodchips, in which the effluent pH was consideredly higher than in the influent and also the highest in all columns which occurred in the pSat columns with woodchips which can be attributed to partial denitrification. This result is in accord with the TN removal in pSat columns with woodchips. The processes of nitrification and partial denitrification reduced the concentration of nitrite and nitrate effectively.

Inorganic Carbon

The presence of woodchips can affect the removal of IC significantly ($p < 0.05$). The removal of IC was positive in uSat and pSat columns without woodchips suggesting the effect of the nitrification process and insignificant effect of denitrification and sulfate reduction. On the other hand, the negative results in the columns with woodchips indicate that there was an insignificant effect of denitrification and sulfate reduction processes on IC removal.

5.3.2 The removal of organic micropollutants in the constructed wetlands

5.3.2.1 Overall results

Most micropollutants were removed significantly with removal efficiency above 50% (Fig. 12.). Five kinds of MPs (CBZ, CAP, MEP, OBZ and TCS) were almost removed completely in the CWs. SUC wasn't detected in all columns. Unsaturated columns with woodchips provided the overall highest removal efficiency of micropollutants as compared to the columns under other conditions. The removal efficiency of most micropollutants in the columns with woodchips was higher than in the columns without woodchips which is due to the adsorption function of woodchips. The removal efficiency of FCZ was only 1% in uSat columns, while the removal efficiency increased significantly in uSat columns with woodchips which was 17% ($p < 0.05$). The removal efficiency of FCZ was higher in pSat columns with woodchips than in

columns without woodchips. The removal efficiency of SMX increased significantly from 61% to 95% under aerobic conditions due to woodchips which overpassed in pSat columns (90% in pSat columns and 93% in pSat columns with woodchips) ($p < 0.05$). Similarly, the removal efficiency of DCF increased from 19% to 82% in uSat columns because of the presence of woodchips. The removal efficiency of TCS in the uSat and pSat columns with woodchips was both higher than in columns without woodchips (Fig.13).

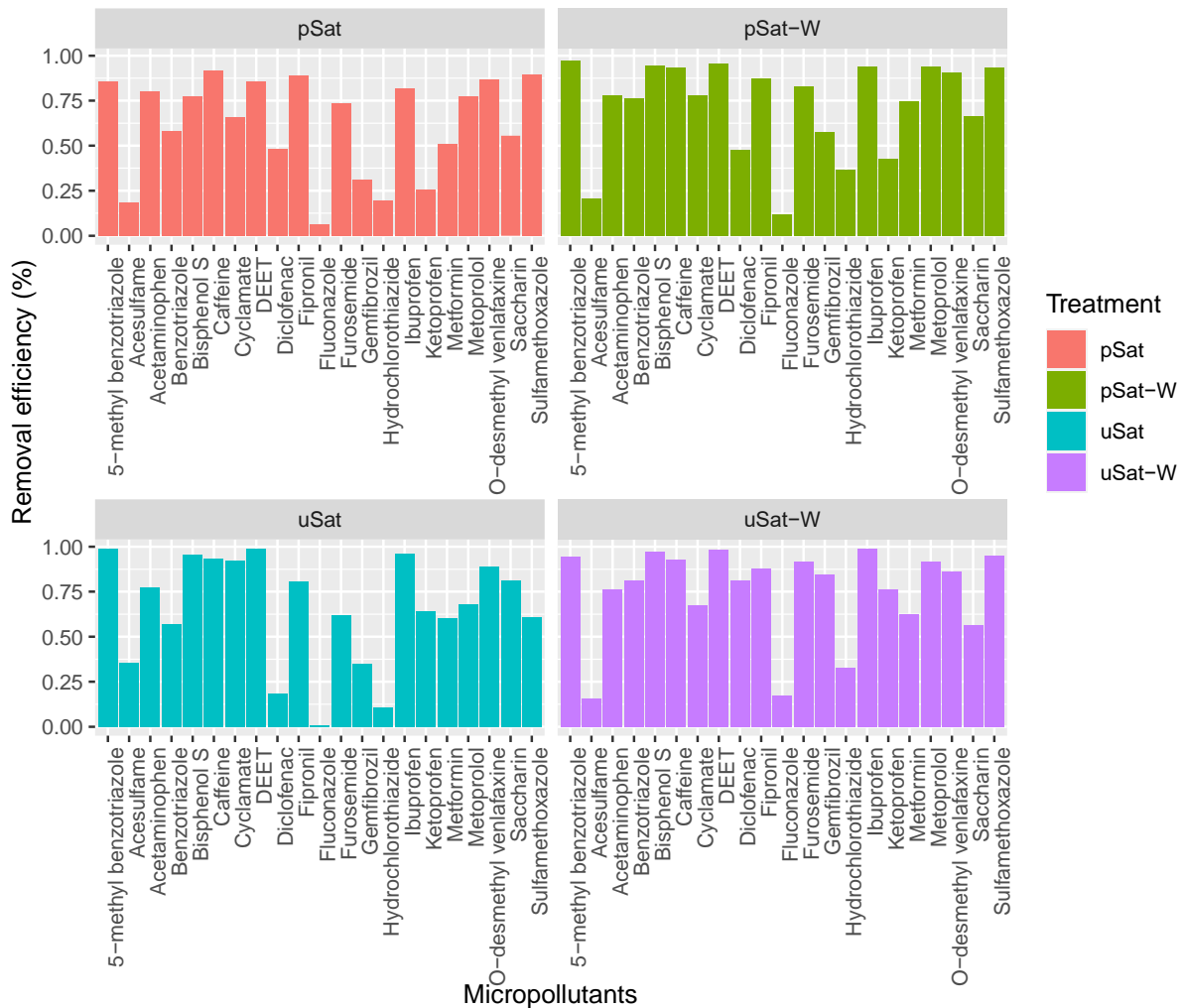


Fig. 12. The removal efficiency of organic micropollutants (pSat-partially saturated columns without woodchips, pSat-W-partially saturated columns with woodchips, uSat-unsaturated columns without woodchips, uSat-W-unsaturated columns with woodchips).

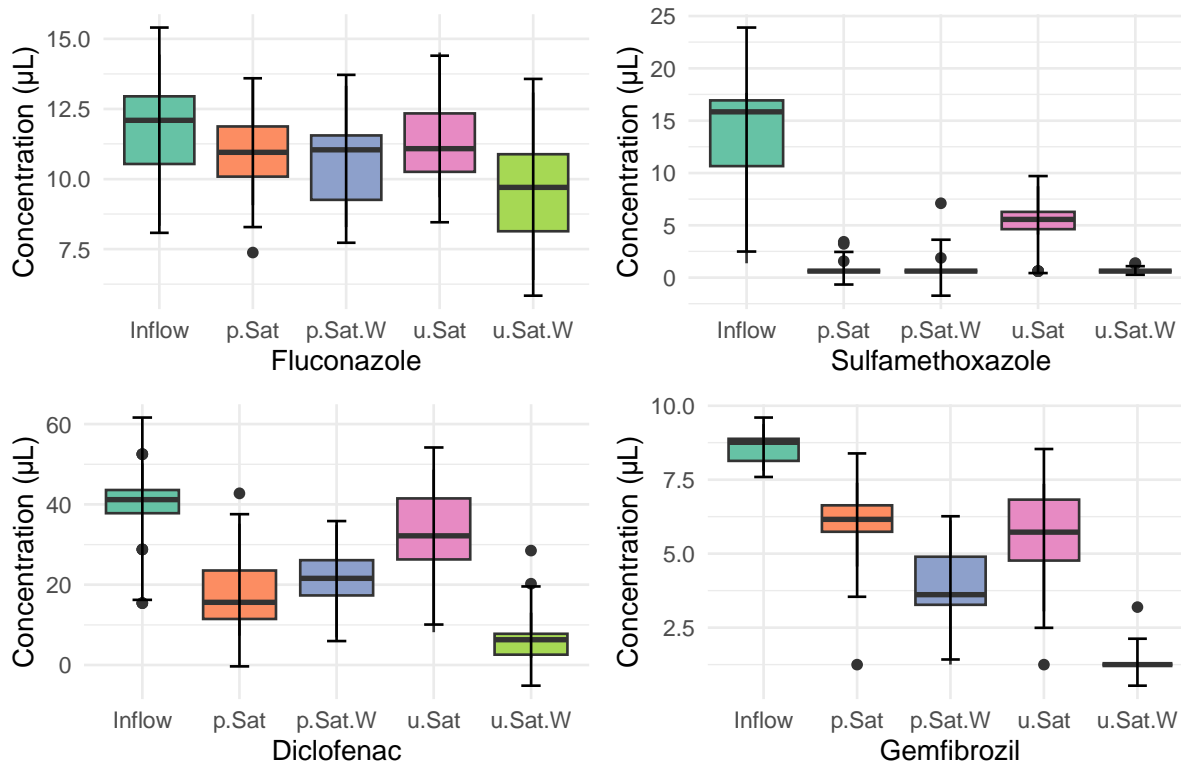


Fig. 13. The concentration (median on bars and MAD as error bars) of the organic micropollutants in the influents and effluents of the experimental columns: fluconazole, sulfamethoxazole, diclofenac and triclosan; data for days 21–85, $n = 8$.

There were three micropollutants (DCF, HCTZ and SMX) removed significantly with woodchips in unsaturated columns ($p < 0.05$). There were six micropollutants (BPS, DEET, FCZ, IBP, MTF and ODV) removed significantly with woodchips in partially saturated columns ($p < 0.05$). However, the removal efficiencies of ASF, FCZ and HCTZ were all under 40% in all columns (Fig. 14& Fig. 15).

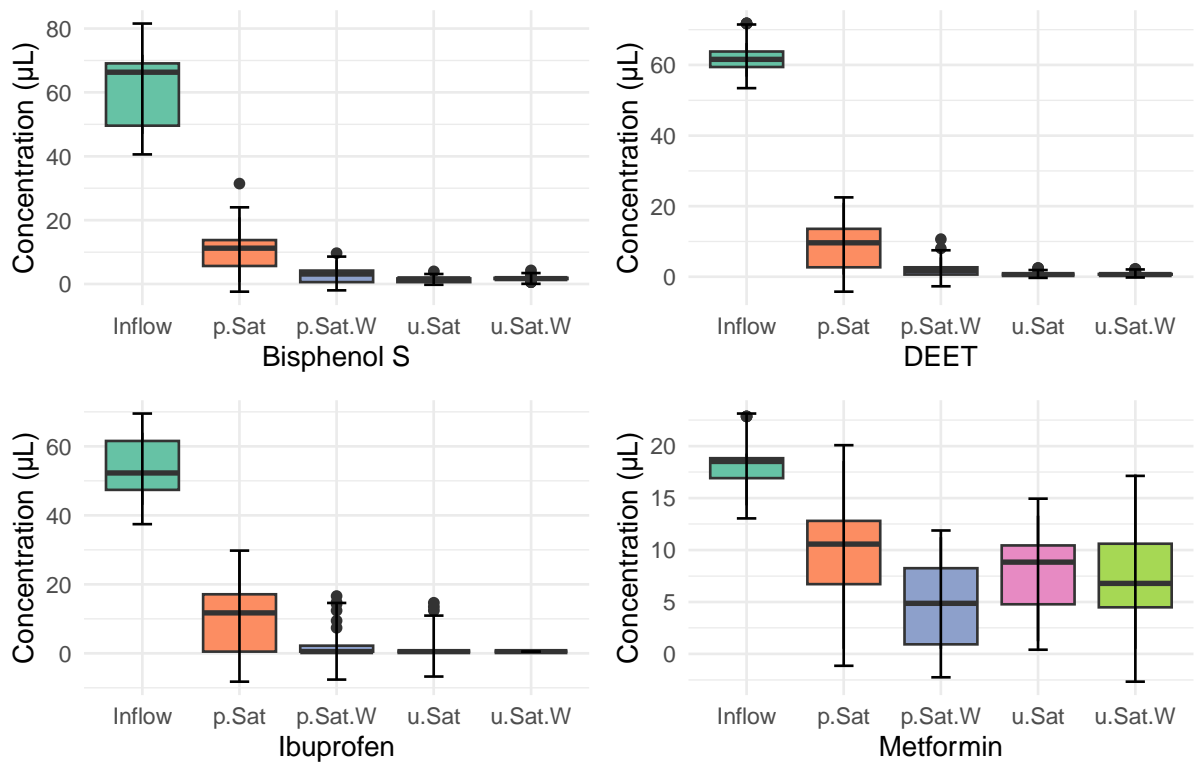


Fig. 14. The concentration (median on bars and MAD as error bars) of the organic micropollutants in the influents and effluents of the experimental columns: bisphenol S, DEET, ibuprofen and metformin; data for days 21–85, $n = 8$.

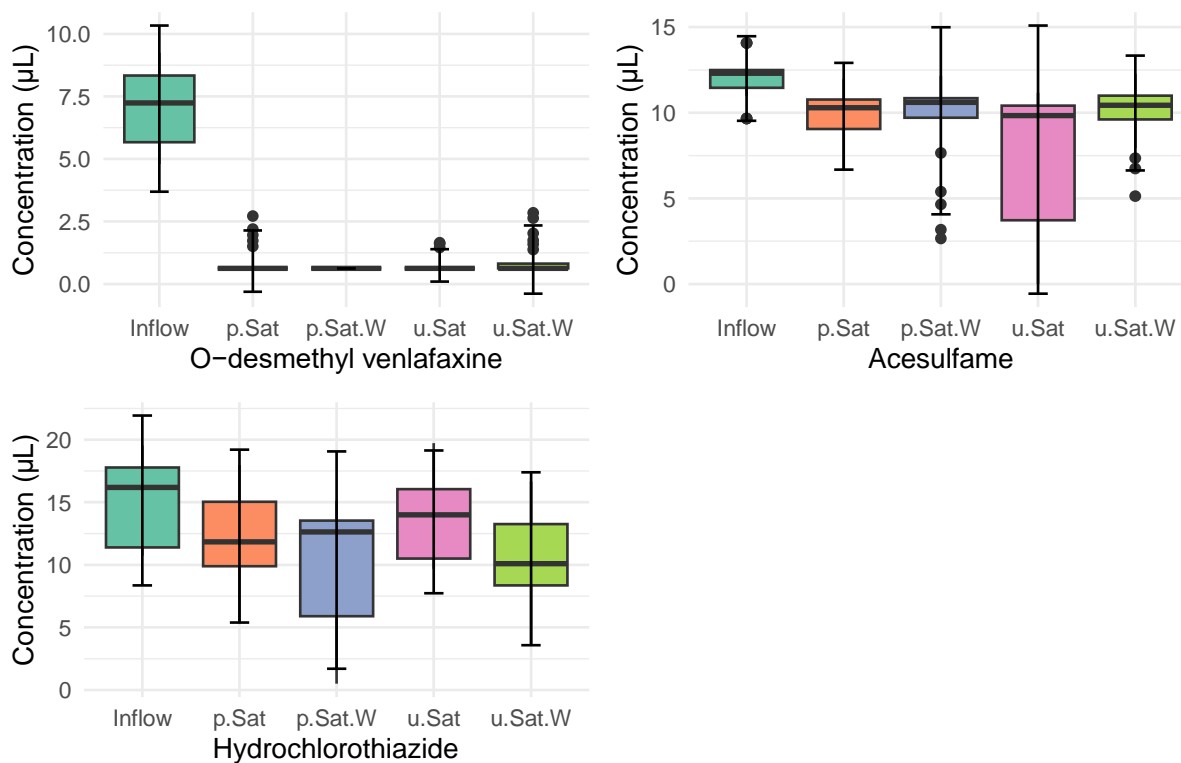


Fig. 15. The concentration (median on bars and MAD as error bars) of the organic micropollutants in the influents and effluents of the experimental columns: o-desmethyl venlafaxine, acesulfame and hydrochlorothiazide; data for days 21–85, n = 8.

5.3.2.2. The impact of woodchips on micropollutants removal

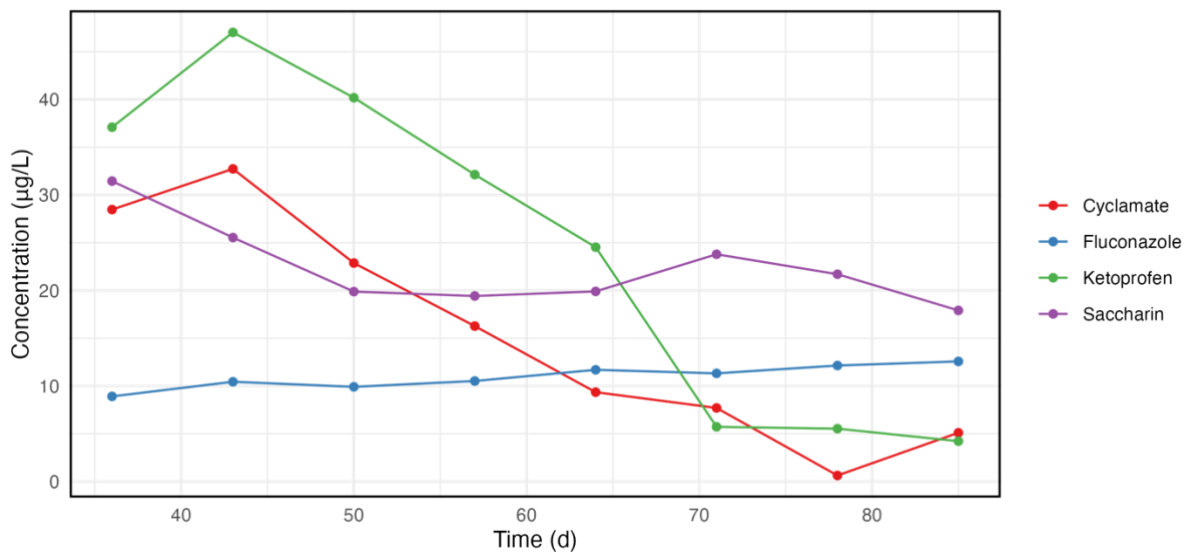
The introduction of woodchips had noticeable effects on various parameters in both influents and effluents. For TN, there was a significant reduction in pSat columns with woodchips effluents compared to pSat influents (39% increase), while uSat columns with woodchips showed an 11% decrease compared to uSat influents. The concentration of N-NO₃ exhibited a substantial decrease in the columns with woodchips, showing that woodchips can enhance the transformation of N-NO₃. N-NH₄ was almost removed in all uSat columns and pSat columns with woodchips, with the removal efficiency of 99.1%, 98.1% and 94.3% separately. TOC demonstrated significant decreases in all woodchip conditions than columns without woodchips, ranging from 89.7% to 95.3% reductions. IC showed varying trends, with minus removal efficiency in some columns because of the artificial addition of woodchips. IC was removed in all columns without woodchips. The concentration of sulfate decreased in columns (ranging from 9% to 16% reduction), while the concentration of phosphate was removed with the assistance of woodchips, with 8% and 42% removal efficiency in the pSat columns and

uSat columns with woodchips. Additionally, the pH value was highest in the pSat columns with woodchips while the situation was the opposite regarding conductivity.

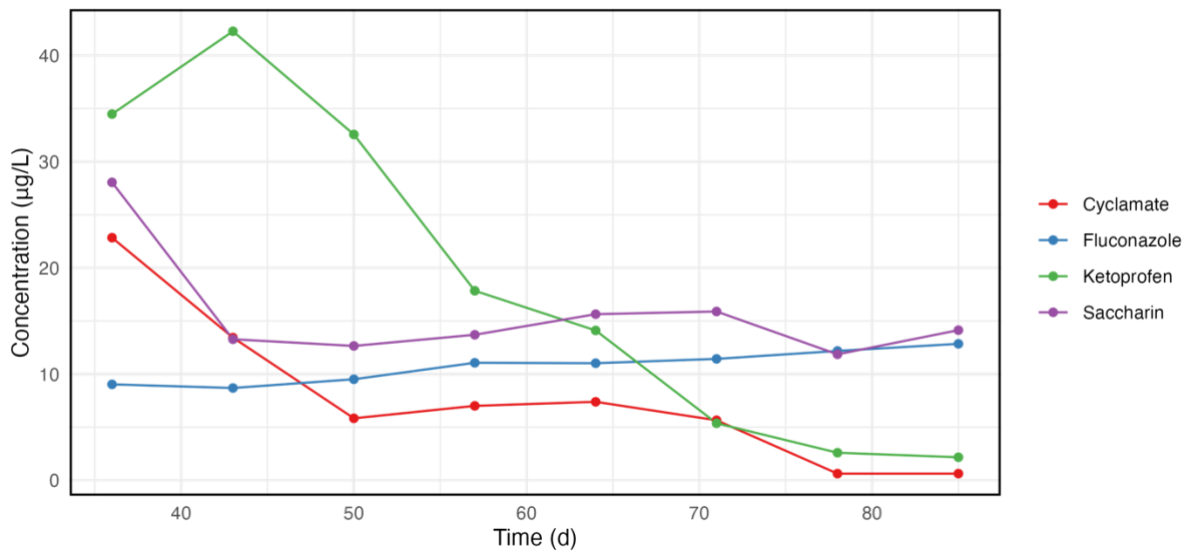
5.3.2.3. Temporal changes in the concentration of micropollutants

The concentration of CLM increased at the beginning and the end of the experiment but it showed a gradual decrease between days 43 to 78 in the pSat columns without woodchips (Fig. 16). In the pSat columns with woodchips, the concentration of CLM showed a decreasing trend from the beginning to the day 78 and then turned stable until the end. The stable situation came earlier in the uSat columns with woodchips which is between day 50 to the end. It may be due to the adsorption sites becoming fully occupied in the columns with woodchips. However, in the uSat columns without woodchips, the concentration of CLM always stayed at a low level because its property of high solubility made CLM loss fast with draining. The concentration of FCZ was also very stable, and it is also stable in other columns. This is because FCZ has low solubility in water and acting as an antifungal agent may reduce the microbial content in the columns. The concentration of FCZ is slightly reduced in the columns with woodchips, it may be due to the adsorption of woodchips, though the effect may be less pronounced due to its antifungal nature.

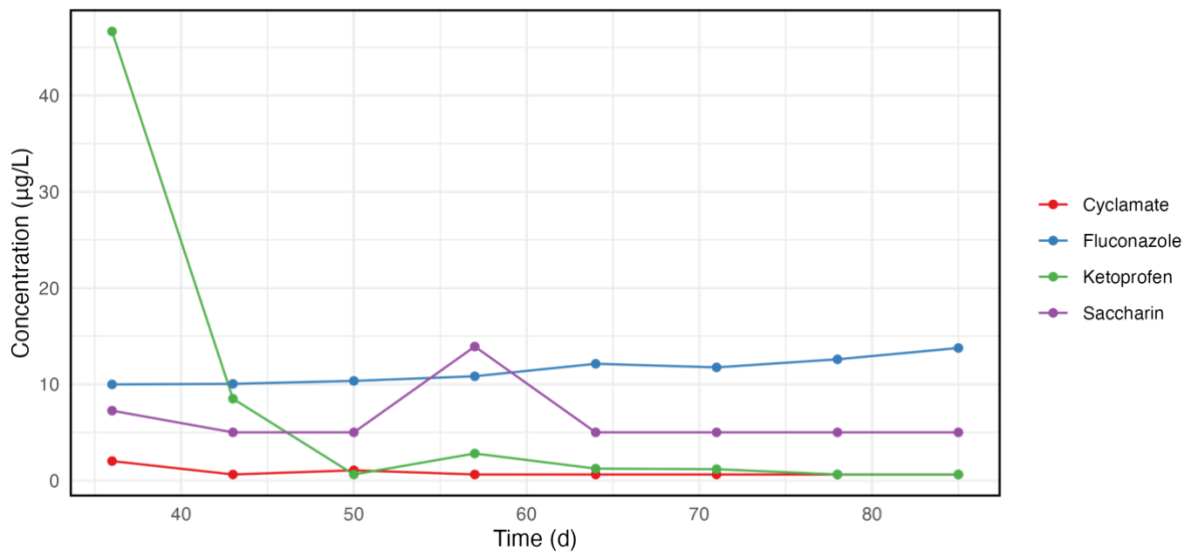
pSat without woodchips



pSat with woodchips



uSat without woodchips



uSat with woodchips

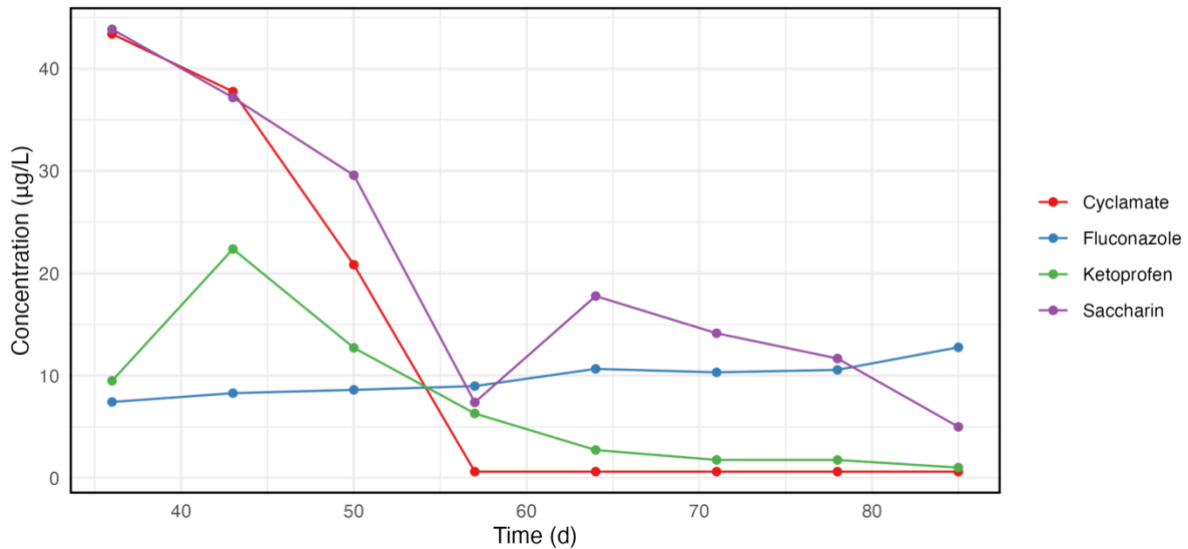


Fig. 16. Time-dependent concentrations of cyclamate, fluconazole, ketoprofen and saccharin in the effluents.

The trend of KTP concentration was similar in all the pSat columns. The concentration of KTP increased from the beginning to the day 43 then decreased almost 50 times between days 43 to 78 and became stable at the end. In the uSat columns, the concentration of KTP turned stable from day 57 to the end. The concentration of SAC rose first and then decreased slightly in the pSat columns without woodchips which was in contrast to the pSat columns with woodchips. The SAC concentration was at a stable level between days 50 to 64 after a decrease from the beginning to the day 50. Then its concentration slightly increased to day 71 but the final concentration decreased. In the uSat columns with woodchip, the overall trend was down. The concentration of SAC was stable between the beginning to the day 50 and between days 64 to the end. However, there was a peak value at the day 57.

6. Discussion

6.1 Experiment No. 1

The obtained results suggest that uSat columns supported microbial aerobic processes of nitrification and organic oxidation, but they cannot provide denitrification or sulfate reduction. With increased water saturation in pSat columns, the nitrification was negligibly affected but the denitrification process was improved, but its efficiency was probably limited by the deficiency of organic carbon. It can be assumed that the water-saturated layer in the pSat columns provided conditions under which the studied organic micropollutants could serve as primary organic carbon sources. The Sat columns provided favourable conditions for partial ammonification-nitrification and denitrification and organic carbon degradation. The columns substantiated also sulfate removal, in contrast to the other columns, which was probably due to the availability of organic carbon and the lack of dissolved oxygen and nitrates, which are more energetically attractive electron acceptors (Chen et al., 2014).

Table 19. illustrates the correlation between effluent quality parameters for all the columns. The FPN effluent concentration has a strong positive correlation with time and a negative correlation with temperature. Then it has a moderate positive correlation with phosphate and TN concentration and a moderate negative correlation with pH and TOC. The KTP effluent concentration has a strong positive correlation with the BPS concentration and a strong positive correlation with N-NH₄, IC and TOC concentrations and a strong negative concentration of sulfate and TN. BPS has a similar correlation pattern as KTP except for the correlation with N-NH₄, TOC and IC, which was very strong. Both BPS and KTP have also a moderate positive correlation with pH.

Table 19. Spearman rank order correlation between the concentrations (or values for pH) of the contaminants in the effluents for all the columns, time (day of sampling) and air temperature (values in bold are significant at $p < 0.05$).

	FPN	KTP	BPS
FPN	1.00	-0.36	-0.29
KTP	-0.36	1.00	0.83
BPS	-0.29	0.83	1.00
N-NH ₄	-0.37	0.77	0.90
TN	0.59	-0.60	-0.62
TOC	-0.42	0.64	0.84
IC	-0.36	0.73	0.82
pH	-0.44	0.40	0.41
Sulfate	0.12	-0.70	-0.82
Phosphate	0.46	-0.10	-0.09
Time	0.74	0.19	0.32
Air temp.	-0.62	-0.15	-0.27

The obtained results indicate that unsaturated CWs play a crucial role in promoting BPS removal and that the removal under saturated conditions can be almost completely hindered. This corroborates the findings of (X. Wang et al., 2019), who investigated the removal of BPS by bacterial consortium enriched from river sediments. That study pointed out that 99% of BPS was promptly degraded under oxic conditions within 10 days. However, some reports exist that state that the removal of BPS under anoxic conditions was superior (Fang et al., 2020; Ike et al., 2006). The degradation pathway of BPS under oxic conditions is started by the impairment of alkyl groups, bound in the two phenolic rings (Ogata et al., 2013). Its aerobic biodegradation may occur through phenolic ring hydroxylation and ring cleavage as reported by (Ogata et al., 2013), who tested *Sphingobium fuliginis* OMI strain isolated from the rhizosphere of aquatic plants. A similar mechanism was also assumed by Kovacic et al. (2021). Given the presence of irrotational double bonds of the sulfonyl group in BPS the compound may be persistent in the biodegradation processes (Fang et al., 2020; Shi et al., 2021).

The behavior of KTP in the present experiment resembled this of BPS. However, the removal was less susceptible to inhibition under reducing conditions as observed for BPS. The degradation of KTP was reported to be initiated in the keto group. (Quintana et al., 2005) denoted the pathway of KTP degradation occurs along the pathway typical of biphenyls, biphenyls ethers and other compounds. The reduction of the KTP group will drive electron density generating reactivity towards electrophilic deoxygenation followed by the formation of

the respective catechol. In short, the hydroxymuconic semialdehyde is formed by meta-cleavage leading to the production of some potential metabolites under subsequent processes (Domaradzka et al., 2015).

Previous studies by (Doran et al., 2009) and (Prado et al., 2021) confirmed that under anoxic conditions, FPN may experience reduction into one of its metabolites namely FPN sulfide whereas in the oxic condition that the metabolite of FPN sulfone would be generated. The removal mechanisms of FPN in CWs can be attributed to adsorption, plant uptake, and biodegradation (Cryder et al., 2021). (Cryder et al., 2021) observed that California bulrush (*Schoenoplectus californicus*) from the natural wetland of Prado Wetlands in South California could accumulate FPN and its metabolites. The study reported the mean concentration of FPN accumulated in the plants was 4.7-194 ng/g, higher than the concentration of the observed metabolites. The moderate correlation of FPN and TN removal suggests the role of denitrifying bacteria in the degradation process. This point is confirmed by a study by (Tomazini et al., 2021) which observed the increasing composition of denitrifiers corresponded to the complete degradation of FPN in anoxic conditions.

BPS and KTP exhibited opposite degradation behaviour against FPN in the CWs. Namely, BPS and KTP were almost completely removed in the unsaturated CWs, but their removal in saturated CWs was only moderate (approx. 50%). The removal of FPN (approx. 90%) was the highest in the saturated and partially-saturate CWs, with moderate removal (66.7%) in unsaturated systems. The effluent concentrations of BPS and KTP were positively correlated with N-NH₄ and TOC concentrations and negatively with TN concentrations, suggesting the crucial role of oxic processes, whereas for FPN inverse correlation was observed implying the key role of anoxic processes. Noteworthy, partially-saturated CWs provided high or very high removal of the studied substances despite their contrary degradability. Namely, their removal efficiencies were 95.9%, 94.5% and 81.6%, for BPS, KTP and FPN, respectively. It can be concluded that partially-saturated CWs can offer high removal efficiency for compounds with contrasting degradation behaviour and can be superior to CWs with either saturated or unsaturated beds. This could be attributed to several factors like higher bacterial diversity and the coexistence of numerous processes responsible for the degradation of compounds of various characteristics.

6.2. Experiment No. 2

The investigation into the removal of pharmaceutical compounds, namely BPS, KTP, DCF, SMX, and FCZ, within various saturated systems has produced nuanced and noteworthy outcomes, shedding light on the complexities of micropollutant behaviour under different filtration conditions. Notably, BPS and KTP showcased high removal efficiency in unsaturated biofilters, underlining the effectiveness of these conditions. The highest removal efficiency of BPS was maintained in saturated biofilters with woodchips, although Sat-Fe exhibited a considerably lower removal efficiency, suggesting the limited impact of iron chips in BPS removal under saturated conditions. In contrast, KTP displayed a diverse range of removal efficiencies, reaching its peak in all unSat columns which was consistent with our previous results (Ren et al., 2023), while registering negative removal efficiency in Sat-CTRL and Sat-Fe-Wch conditions. This variability emphasizes the sensitivity of KTP removal to specific filtration settings. On the other hand, the investigation revealed negligible removal of DCF, SMX, and FCZ in unsaturated columns, categorizing FCZ and DCF as recalcitrant compounds. Anoxic processes exhibited potential for DCF removal in saturated columns, albeit with a modest efficiency similar to the trend observed for FCZ (Liu et al., 2018). The removal patterns of SMX and BPS were notably distinct, with SMX initially proving recalcitrant in unsaturated columns but being completely removed in saturated conditions, particularly in the presence of iron chips (Sat-Fe and Sat-Fe-Wch). This indicated that absorption may be the effective way to remove SMX, especially when coupled with iron chips (Kang et al., 2018).

Luminescence inhibition assays provided additional insights, demonstrating a significant decrease in SMX luminescence inhibition in columns with iron chips and woodchips, suggesting potential improvements in water quality. Furthermore, negative luminescence inhibition (luminescence stimulation) in columns with iron chips and woodchips, mirroring the trend observed with SNM, raises questions about the potential environmental implications of SNM. The luminescence inhibition of ISX consistently showed negative values in both influents and effluents, indicating its persistent characteristics. Ecotoxicity assessments revealed a weak correlation between SMX and *Aliivibrio fischeri*, suggesting that SMX's inherent toxicity may be overshadowed by more toxic transformation products. The addition of iron chips significantly enhanced the degradation of SMX, as evidenced by the removal efficiency and luminescence inhibition results. However, the persistence of SNM, identified as

a transformation product of SMX, raises environmental concerns, as it may not undergo further degradation. The tentative identification of six transformation products of SMX, with TP6 involving the formation of SNM, suggests a common cleavage pattern in all types of columns. The superiority of iron chips in SMX removal may be attributed to greater reaction kinetics, indirectly indicated by the presence of SNM (TP6). Nevertheless, the negligible removal of SNM raises concerns about its potential environmental risk, as it may pose a persistent threat. Moreover, SNM are prone to experience reversible inter-transformation with their respective metabolites. This phenomenon could explain the observed negative removal of certain SNM, like SMX, where the apparent removal efficiency might be influenced by the transformation dynamics between the parent compound and its metabolites (Dan A et al., 2013). This underscores the intricacies of micropollutants removal processes and the necessity for a thorough understanding of both parent compounds and their transformation products to develop effective water treatment strategies and safeguard aquatic ecosystems.

6.3. Experiment No. 3

The results of this study suggest that unsaturated columns can be beneficial for nitrification but a limited condition for denitrification. Partially-saturated columns could improve this weakness by increasing water levels. In our previous study, TN removal was limited by organic carbon sources. In this way, woodchips were added as the supply. It could be assumed that nitrification occurred in unsaturated water layers where organic micropollutants served as primary carbon sources. Furthermore, denitrification occurred in saturated water layers where partial ammonification-nitrification and organic carbon degradation were also presented. In environments with limited oxygen, nitrite can be directly converted to nitrous oxide and/or dinitrogen gas through denitrification, without the intermediate step of being transformed into a nitrate (Vymazal, 2007). Woodchips added in the columns supported organic micropollutant removal and denitrification as a necessary supplementary carbon source for the process. Woodchips in the columns could enlarge the surface of the substrate for adsorbing more micropollutants and the growth of microorganisms (Tejedor et al., 2020; Yuan et al., 2020) which should be another reason for the increased removal efficiency. The elimination of MPs is thought to result from a process that includes both the absorption on the surface areas of sand and woodchips and their absorption into biofilms that develop on these substrates. This removal process is primarily reliant on each compound's sorption capacity but is also affected by the surface characteristics and electric charge of the materials, as well as biofilm presence. Future studies will further elucidate the specific impact of woodchips on the absorption and overall removal of MPs. Fig. 17. shows the correlation between parameters of effluent in all columns. The TN effluent removal efficiency has a strong positive correlation with N-NO₃ and Phosphate and a negative correlation with TOC, TC and IC. The TN effluent removal efficiency has a moderate positive correlation with conductivity and a moderate negative correlation with sulfate, N-NH₄ and N-NO₂.

Fig. 18. illustrates the correlation between effluent quality parameters for all the columns. The TN removal efficiency has a strong positive correlation with HCTZ, MTP, BTA, SMX, FCZ, FUR, DCF, MTF, CFE, TCS and N-NO₃ effluent concentration and a negative correlation with TOC, TC and IC. Then it has a moderate positive correlation with BPS, FPN and ODV concentration and a moderate negative correlation with SAC, CLM, 5-MEBT and pH. The obtained results indicate that unsaturated CWs play a crucial role in promoting 5-MEBT, BPS, CFE, CLM, DEET, IBP, MTP and SMX removal. In the columns with woodchips, the removal

efficiency of DCF, HCTZ and SMX increased significantly in unsaturated water level columns. The obtained results indicate that partially-saturated CWs play a crucial role in 5-MeBT, APAP, CFE, DEET, FPN, IBP, ODV and SMX. Woodchip can improve the removal efficiency of BPS, DEET, FCZ, IBP, MTF and ODV significantly in partially-saturated columns. The removal of CLM showed an increasing overall trend in the tested CWs, which was different from the previous stable property. The degradation mechanism of CLM needs to be further explored by the evolution of genes or the effect of microorganisms.

Previous studies indicated that SMX was resistant due to the presence of sulfur which can hinder the microbiological degradation (Bertelkamp et al., 2014). IBP, despite not being constrained by its structure in terms of biodegradation, exhibits significant variations in degradation when subjected to aerobic and anaerobic conditions (Tseng et al., 2020). The studies by Wang et al., 2019) and Suarez et al., 2010) found that the removal efficiency of IBP was almost removed completely under aerobic conditions (>90%); however, under anaerobic conditions, the removal efficiency was limited. In our study, the removal of IBP was increased significantly from 82% to 94 % with the addition of woodchips ($p < 0.05$). In this work, woodchips tended to improve micropollutants removal which indicated a crucial function of woodchips under conditions where oxygen supplements are sufficient. However, the results in this work corresponded with previous studies investigating the biodegradation of SMX, BTA and 5-MeBT; these four compounds showed efficient biodegradation in unsaturated and partially-saturated systems, especially with woodchips which are different from previous studies (Dong et al., 2016; Liu et al., 2011).

The concentration of phosphate increased in unsaturated and partially-saturated columns without woodchips. Phosphate removal was regarded as a challenge in the field of wastewater treatment (Martínez et al., 2018). However, phosphate was removed significantly in the columns with woodchips compared to the columns without woodchips in this study ($p < 0.05$). There was a higher removal efficiency in unsaturated columns with woodchips. The mechanisms of phosphate removal by the woodchip may be because of several aspects. Firstly, microbial growth is better due to more oxygen content in unsaturated columns within the bioreactor would deplete a large number of the phosphate. Secondly, the extracellular polymeric substances produced by the biofilm on the woodchips may adsorb phosphate (Li et al., 2015). Thirdly, phosphate may also be adsorbed by woodchips from the influent. Biofilm

penetration and microbial degradation of woodchips may be important in developing phosphate removal capability (Cameron and Schipper, 2010).

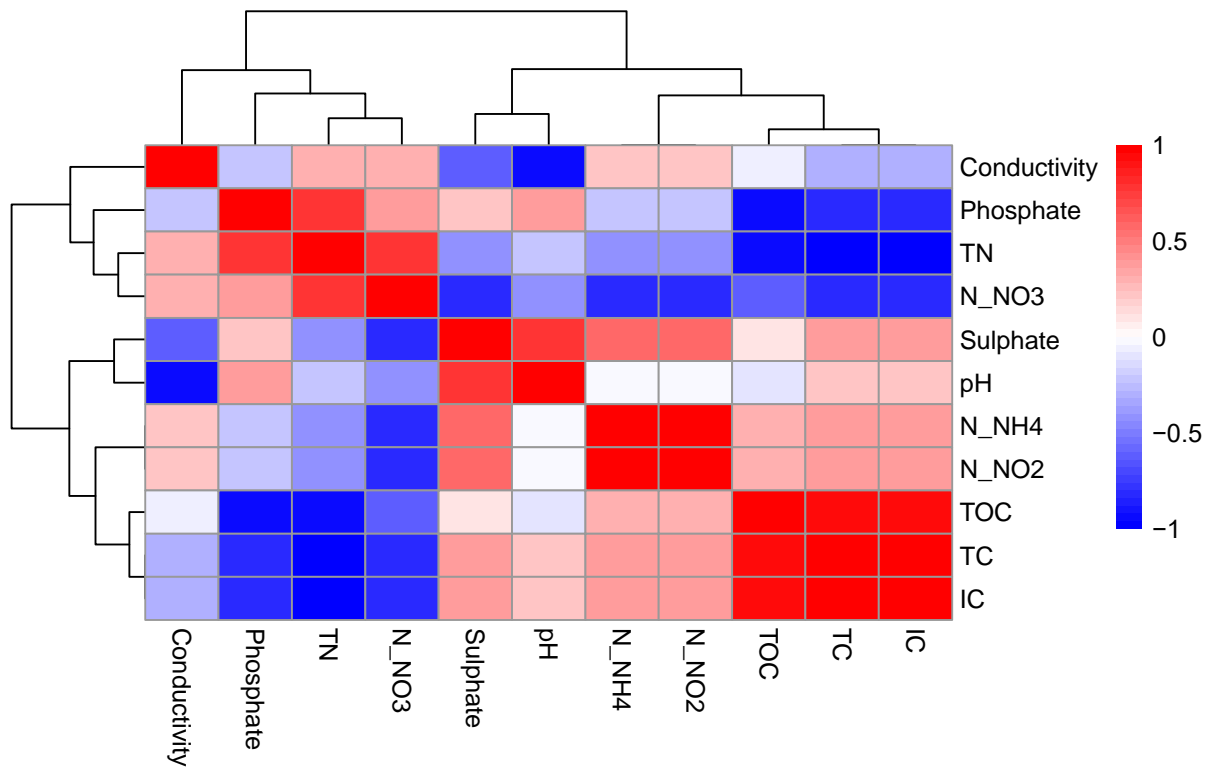


Fig. 17. Spearman rank order correlation among the removal efficiency of the parameters in the effluents for all the columns.

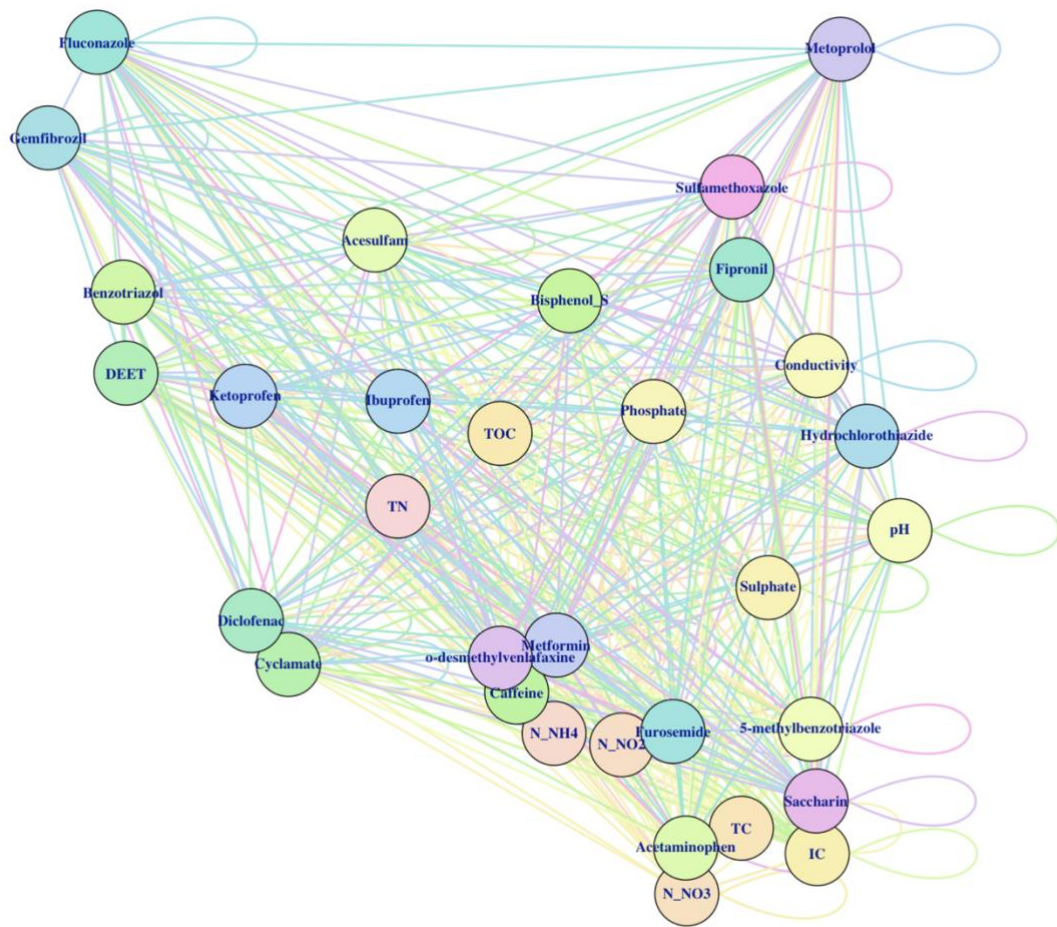


Fig. 18. Spearman rank order correlation between the removal efficiency of all micropollutants and standard parameters in the effluents for all the columns.

7. Conclusions

7.1 Experiment No. 1

The CWs used in this experiment offered high removal efficiency for BPS, KTP, and FPN, but the removal efficiency depended considerably on the water saturation level. BPS and KTP exhibited contrasting behaviour against FPN in the CWs in the present experiment. Under saturated conditions, the removal of BPS and KTP was only moderate and considerably less efficient than under unsaturated conditions. However, the FPN removal efficiency was the highest (approx. 90%) under partially-saturated or saturated conditions. Interestingly, the removal of all three tested compounds in partially-saturated CWs was at the same or slightly lower level compared with the best treatment option. The studied partially-saturated CWs maintained a very high removal efficiency of N-NH₄ (99.4%-99.6%) and TOC (above 84%) that was comparable with that in the unsaturated CWs. This allows considering partially-saturated CWs as a specific performance all-rounder for micropollutants with contrary degradation characteristics, which is an attractive alternative to hybrid CWs like a combination of at least two steps, e.g. vertical-flow bed and a horizontal-flow bed. The existing unsaturated vertical-flow CWs can be easily retrofitted at a minimum cost to turn into partially-saturated CWs.

7.2 Experiment No. 2

This study was conducted on the degradation of SMX, ISX and SNM under different water-saturated levels. Notably, the introduction of iron chips significantly enhanced the degradation of SMX (100% in Sat-Fe and Sat-Fe-Wch columns), showcasing improved remediation capabilities. Conversely, SNM exhibited persistence across various conditions, suggesting resistance to degradation processes. ISX, on the other hand, demonstrated complete degradation in all types of columns utilized. Despite this successful removal, determining the cleavage site of the molecule remained elusive without further analysis, specifically through MS/MS. The toxicity assessments revealed that SMX inhibited plant indicator organisms, with its toxicity correlating with the removal process. In contrast, ISX inhibited bacteria indicator organisms, and its toxic effects persisted even after efficient removal, highlighting a unique challenge in managing its environmental impact.

7.3 Experiment No. 3

The CWs used in this experiment offered high removal efficiency for 5-MeBT (86-99%), BPS (78%-97%), CFE (92%-93%), DEET (86%-99%), FPN (81%-89%), IBP (82%-99%), ODV (86%-91%) and SMX (61%-95) but the removal efficiency depended considerably on the water saturation level and the presence of woodchips. APAP, HCTZ and MTP can be removed more in the partially-saturated CWs than in the unsaturated CWs under the same substrate conditions. The situation of BPS, DEET, GFRZ, IBP and KTP is in contrast. TTA, ASF, BTA, BPS, CFE, CLM, DEET, FCZ, FUR, GFRZ, HCTZ, IBP, KTP, MTF, MTP, SAC and SMX have higher removal efficiency with the presence of woodchips in the partially-saturated CWs contrasted with APAP and FPN. BTA, BPS, DCF, FPN, FCZ, FUR, GFRZ, HCTZ, IBP, KTP, MTF, MTP and SMX can be removed better in the unsaturated CWs with woodchips than the unsaturated CWs without woodchips. TTA, ASF, APAP, CLM, DEET, ODV and SAC can be removed more in the unsaturated CWs without woodchips. The removal efficiency of FCZ and ASF was lower than 20% and 40% separately in all columns that were persistent. However, CBZ, CAP, MEP, OBZ, SUC and TCS were almost all removed under all conditions. Interestingly, the studied partially-saturated CWs with woodchips maintained a very high removal efficiency of TN (46%), in sharp contrast to the CWs with unsaturated water levels (4%-15%). This allows consideration of partially-saturated CWs with woodchips as a good choice for TN removal and woodchips can also increase the removal efficiency of MPs considerable to unsaturated CWs for most of the MPs. Woodchips played a key role for most MPs removal.

7.4 Final remarks

In conclusion, CWs employed in these three studies demonstrated high removal efficiency for a range of micropollutants, including SMX, DCF, FPN, KTP, CBZ, BPS, MTP, BTA, 5-MeBT, ASF, APAP, CFE, CLM, DEET, IBP, OBZ, SAC, FUR, MTF, HCTZ, ODV, TCS, MEP, GFRZ and CAP. The efficiency, however, was found to be contingent on water-saturated levels and the presence of woodchips. Notably, partially-saturated CWs, either with or without woodchips, emerged as versatile performers, especially achieving the highest removal efficiency of TN (46%) indicated the best treatment option of nitrogen removal. The addition of iron chips significantly enhanced the degradation of SMX, improving remediation capabilities, while SNM exhibited persistence under different conditions. ISX was completely degraded but posed challenges in determining the cleavage site. Toxicity assessments revealed intriguing correlations, such as SMX inhibiting plant indicator organisms and ISX persisting in toxic effects even after efficient removal. Overall, this study suggests that woodchips can increase the removal of most MPs in both partially-saturated and unsaturated CWs, present an attractive and cost-effective alternative for micropollutants removal, outperforming traditional WWTPs configurations.

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Curriculum vitae& List of publications

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Thesis: *The influence of forest fire interference on nitrogen mineralization in Daxingan Ridge*

Publications

Ren, T., Perdana, M.C., Kříženecká, S., Sochacki, A., Vymazal, J. 2023. Constructed wetlands for the treatment of household organic micropollutants with contrasting degradation behaviour: Partially-saturated systems as a performance all-rounder. *Chemosphere* 314, 137645. <https://doi.org/10.1016/j.chemosphere.2022.137645>

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Submitted articles and manuscripts in preparation

Perdana, M.C., Sochacki, A., Çömez, F.O., Silva, Adrián M.T., Tiritan, M.E., Berchová, Kateřina., Chval, Z., Lexa, M., **Ren, T.**, Beesley, L., Ribeiro, A.R.L., Vymazal, J. (2023) The Resilience of Nature-Based Systems Treating Greywater: The Effect of Operating Conditions and Seasonal Temperature Decline. Manuscript submitted to *Journal of Water Process Engineering*.

Grants and projects

- Co-worker on grant: SWAMP (Project responsible water management in built-up areas in relation to the surrounding landscape).
CZ.02.1.01/0.0/0.0/16_026/0008403 (Czech Science Foundation)
- Co-worker on grant: Grow safely- do not support invasive species.
NO. 3211100006 (Czech Science Foundation)
- Principal investigator on grant: The relationship between the process of PPCPs degradation and nitrogen removal and its effect on microbial community structures in constructed wetlands (CWs): triclosan and sulfonamides as model contaminants.
IGA 2020B0026 (Czech University of Life Sciences Prague)
- Principal investigator on grant: The mechanism of removal of Pharmaceuticals and personal care products (PPCPs) in constructed wetlands (CWs) under aerobic and anaerobic conditions.
IGA 2022B0028 (Czech University of Life Sciences Prague)

Participation in conferences

11.2019	Kostelecké inspirování 2019, Prague, Czech Republic
03.2021	12th International Conference on Environmental Science and Development (Online)
06.2022	3rd International Scientific Conference Ecological and Environmental Engineering, Poznan, Poland
06.2023	3rd International Meeting on New Strategies in Bioremediation/Restoration Processes, Muttenz, Switzerland
09.2023	10th WETPOL (Wetland Pollutant Dynamics and Control) 2023, Bruges, Belgium