

Czech University of Life Sciences Prague

Faculty of Environmental Sciences

Department of Applied Ecology



Diploma Thesis

**Impact of substrate adsorption behaviour for
emerging pollutants in constructed wetlands**

Author: Yana Prados

Supervisor: doc. Dr.-Ing. Zhongbing Chen

Consultant: Ph.D. candidate Bo Hu



Czech University of Life Sciences Prague
Faculty of Environmental Sciences

DIPLOMA THESIS TOPIC

Author of the thesis: Yana Prados
Study program: Landscape Engineering
Field of study: Landscape Planning
Thesis supervisor: doc. Dr.-Ing. Zhongbing Chen
Supervising department: Department of Applied Ecology
Language of a thesis: English

Thesis title: **Impact of substrate adsorption behaviour for emerging pollutants in constructed wetlands**

Objectives of the thesis: (1) Assess the adsorption capacity of different substrates for selected emerging pollutants.
(2) Evaluate the effects of substrates' adsorption capacity on emerging pollutants removal in constructed wetlands.

Methodology: Six vertical subsurface flow CWs will be established at the Czech University of Life Sciences Prague. The experimental device consisted of the innovative KG-System (PVC) pipes, substrate, and water outlet. The 6 PVC pipes will be established to simulate the subsurface flow CWs with the dimensions of each system is 15 × 55 cm (diameter × Height). *Glyceria maxima* will be selected as an experimental plant. EPs will include ibuprofen and diclofenac. At the end of this experiment, the rhizosphere substrates will be collected and placed in a 40 °C oven for 120 h to prepare a dry sample.

The proposed extent of the thesis: 50

Keywords: Emerging pollutants; Pharmaceutical and personal care products; Constructed wetlands; Adsorption; Substrates.

Recommended information sources:

1. Asher Bar-Tal, ... Markus Tuller, in *Soilless Culture (Second Edition) 2019: Inorganic and Synthetic Organic Components of Soilless Culture and Potting Mixture*
2. Brix, H.; Arias, C. A. (2005): *The Use of Vertical Flow Constructed Wetlands for on-Site Treatment of Domestic Wastewater: New Danish Guidelines*. In: *Ecological Engineering*.
3. D.Herskovitch and I.J.Lin Mineral Engineering Research Centre, Technion, Haifa 32000, Israel (1995): *Upgrading of raw perlite by a dry magnetic technique*
4. H.C.Tee, C.E.Seng, A.Md.Noor, P.E.Lim: *Performance comparison of constructed wetlands with gravel- and rice husk-based media for phenol and nitrogen removal*
5. Cheng Dong, Mengting Li, Lin-Lan Zhuang *, Jian Zhang *, Youhao Shen and Xiangzheng Li: *The Improvement of Pollutant Removal in the Ferric-Carbon Micro-Electrolysis Constructed Wetland by Partial Aeration*
6. UN-HABITAT (2008): *Constructed Wetlands Manual*. Kathmandu: UN-HABITAT, Water for Asian Cities Program.
7. Yan Yang, Yaqian Zhao, Ranbin Liu: *Global development of various emerged substrates utilized in constructed wetlands*

Expected date of thesis defence: 2021/22 SS - FES

Recognition

I would like to express my heartfelt gratitude and deepest appreciation to my thesis supervisor, doc. Dr.-Ing. Zhongbing Chen, of the Faculty of Environmental Sciences at Czech University of Life Sciences. I am also very thankful to Ph.D. candidate Bo Hu and his assistance in keeping my progress on schedule and his constructive advice by steering me in the right direction.

Declaration

I hereby declare that I have independently elaborated the diploma/final thesis with the topic "Impact of substrate adsorption behaviour for emerging pollutants in Constructed Wetlands." I have cited all the information sources listed at the end of the thesis in the list of used information sources.

I am aware that my diploma/final thesis is subject to Act No. 121/2000 Coll., on copyright, on rights related to copyright, and on the amendment of some acts, as amended by later regulations, particularly the provisions of Section 35(3) of the act on the use of the thesis.

By submitting the diploma/final thesis, I am aware that I agree with its publication under Act No. 111/1998 Coll., on universities and the change and amendments of some acts, as amended, regardless of the result of its defense.

With my signature, I declare that the electronic version is identical to the printed version, and the data stated in the thesis has been processed concerning the GDPR.

Yana Prados 2021

Abstract

Emerging pollutants (EPs) have become a considerable concern for human health and all biota, thereby endangering the health and lives of the whole environmental system. Pharmaceutically active compounds, including over-the-counter medications, have more frequently been found throughout the water bodies around the world. Moreover, a pandemic caused by the SARS-CoV-2 virus has entailed increasing the use of various chemicals and compounds, including Pharmaceuticals and Personal Care Products. Therefore, the risk of environmental damage has received extensive attention in recent years.

This research aims to investigate, evaluate, and compare the role of two different substrates, sand, and perlite, within the waste products removal process regarding the adsorption capability of EPs, including ibuprofen (IBU) and diclofenac (DCF) in constructed wetlands (CWs).

The comparative results showed that perlite provides a superior condition for plant growth versus sand and indicates that the size difference of the plant shoots and roots length is 20% and 16%, respectively, in favor of perlite. In addition, the removal efficiencies of TOC, PO_4^{3-} and NH_4^+ have shown the best sorption results using perlite increased treatment process by 5%, 25%, and 42%, respectively, compared to sand. Furthermore, the influence of perlite also contributed to higher IBU and DCF removal efficiency. It was 88.57% and 63.48% for perlite, which is higher than the adsorption ability of sand by 23 and 27%, respectively. Besides, the perlite significantly boosts the contents of IBU in the rhizosphere soil and raises the presence of DCF in plant roots. Moreover, the contents of IBU and DCF metabolites (2-OH IBU and 4'-OH DCF) in the plant roots also was higher.

It can be concluded that perlite may be contributing to the high removal efficiency of emerging pollutants, including pharmaceuticals. Thus, the characteristics of this substrate are promising due to its effectiveness for emerging pollutants removal. This paper considers the components, pathways, and impact of pollutants on the ecosystem. The presumed mutual influence and co-dependency of the elements of nature can shed new light on the existing problem and may contribute to solving it.

Keywords: Emerging pollutants; Pharmaceutical and personal care products; Constructed wetlands; Adsorption; Substrates.

Abstract

Vznikající polutanty (EP) se staly značným problémem pro lidské zdraví a celou biotu, čímž ohrožují zdraví a životy celého systému životního prostředí. Farmaceuticky aktivní sloučeniny, včetně volně prodejných léků, byly častěji nalezeny ve vodních útvarech po celém světě. Pandemie způsobená virem SARS-CoV-2 si navíc vyžádala zvýšené používání různých chemikálií a sloučenin, včetně léčiv a produktů osobní péče. Proto je v posledních letech věnována velká pozornost riziku poškození životního prostředí. Tento výzkum si klade za cíl prozkoumat, vyhodnotit a porovnat roli dvou různých substrátů, písku a perlitu, v procesu odstraňování odpadních produktů, pokud jde o adsorpční schopnost EP, včetně ibuprofenu (IBU) a diklofenaku (DCF) ve vybudovaných mokřadech (CWs). Srovnávací výsledky ukázaly, že perlit poskytuje lepší podmínky pro růst rostlin oproti písku a ukazuje, že velikostní rozdíl v délce výhonků a kořenů rostlin je 20 % a 16 % ve prospěch perlitu. Kromě toho účinnost odstraňování TOC, PO_4^{3-} a NH_4^+ prokázala nejlepší sorpční výsledky při použití perlitu se zvýšeným procesem úpravy o 5 %, 25 % a 42 % v porovnání s pískem. Kromě toho vliv perlitu také přispěl k vyšší účinnosti odstraňování IBU a DCF. U perlitu to bylo 88,57 % a 63,48 %, což je o 23, resp. 27 % vyšší než adsorpční schopnost písku. Kromě toho perlit významně zvyšuje obsah IBU v půdě rhizosféry a zvyšuje přítomnost DCF v kořenech rostlin. Kromě toho byl také vyšší obsah metabolitů IBU a DCF (2-OH IBU a 4'-OH DCF) v kořenech rostlin. Lze dojít k závěru, že perlit může přispívat k vysoké účinnosti odstraňování vznikajících znečišťujících látek, včetně léčiv. Vlastnosti tohoto substrátu jsou tedy slibné díky jeho účinnosti při odstraňování vznikajících znečišťujících látek. Tento článek se zabývá složkami, cestami a dopadem znečišťujících látek na ekosystém. Předpokládané vzájemné ovlivňování a spoluzávislost přírodních živlů může vrhnout nové světlo na existující problém a může přispět k jeho řešení.

Klíčová slova: Emerging pollutants; Farmaceutické výrobky a výrobky pro osobní péči; Vybudované mokřady; Adsorpce; Substráty.

List of Abbreviations

| | |
|--------|--|
| CWs | Constructed Wetlands |
| DCF | Diclofenac |
| GW | Greywater |
| IBU | Ibuprofen |
| EPs | Emerging Pollutants |
| HSSF | Horizontal Subsurface Flow |
| NSAIDs | Non-Steroidal Anti-Inflammatory Drugs |
| N | Nitrogen |
| P | Phosphorus |
| pH | Potential of Hydrogen |
| PPCPs | Pharmaceuticals and Personal Care Products |
| PVC | Polyvinyl Chloride |
| TN | Total Nitrogen |
| TOC | Total Organic Carbon |
| VSSF | Vertical Subsurface Flow |
| WWTPs | Wastewater Treatment Plants |

Table of Contents

| | |
|--|----|
| List of Abbreviations..... | 7 |
| List of tables | 9 |
| List of figures..... | 9 |
| 1. Introduction..... | 1 |
| 2. Literature Research..... | 3 |
| 2.1 Emerging pollutants..... | 3 |
| 2.2 Ecotoxicity of EPs | 6 |
| 2.2.1 Synergistic toxic effects | 8 |
| 2.2.2 Removal efficiency in wastewater treatment plants..... | 8 |
| 2.3 Pharmaceuticals in the environment: Ibuprofen and Diclofenac | 11 |
| 2.4 Constructed Wetlands | 15 |
| 2.5 EPs removal Mechanisms in Constructed Wetlands..... | 18 |
| 2.5.1 Biodegradation process | 19 |
| 2.5.2 Phosphorus and Nitrogen removal in CWs | 20 |
| 2.5.3 Sorption: an overview. Role of the substrate in the sorption process | 23 |
| 2.5.4 Importance of plants in CWs..... | 26 |
| 3. Objectives | 28 |
| 4. Method and materials | 29 |
| 4.1 Experimental setup | 31 |
| 4.2 Sample analysis | 33 |
| 4.2.1 Water quality parameters | 33 |
| 4.2.2 Analysis of IBU and DCF and their metabolites in CWs..... | 35 |
| 5. Results | 36 |
| 5.1 Plant biomass | 36 |
| 5.2 Wastewater parameters | 38 |
| 5.3 Ibuprofen and Diclofenac in the effluent | 44 |
| 5.4 Ibuprofen and Diclofenac in the plant roots and rhizosphere soil and plant roots | 46 |
| 6. Discussion..... | 48 |
| 7. Conclusions..... | 53 |
| 8. Bibliography | 54 |

List of tables

| | |
|--|----|
| Table 1. The occurrence of DCF in various environmental compartments worldwide. Copyright (Sathishkumar et al., 2020) | 14 |
| Table 2. The concentration of nutrients in wastewater solution prepared to simulate municipal sewage (mg L^{-1})..... | 32 |
| Table 3. Physical-chemical capacities of pharmaceuticals. Source https://pubchem.ncbi.nlm.nih.gov/ | 32 |
| Table 4. The replication phases during the five-month study period | 33 |
| Table 5. The measurement stages for chemical compound and indicator of water quality | 36 |
| Table 6. Ibuprofen and Diclofenac content and their metabolites in the rhizosphere soil ($\mu\text{g/kg}$)..... | 46 |

List of figures

| | |
|--|----|
| Figure 1. Source and pathways of EPs in the environment. Created by author, 2021 | 3 |
| Figure 2. Schematic pathways of some EPs from sources to receptors, Copyright (Stuart et al., 2012)..... | 5 |
| Figure 3. Transformation pathways of different pharmaceuticals in the environment. Copyright (Patel et al., 2019)..... | 13 |
| Figure 4. Types of CWs for wastewater treatment. Created by the author (2021) | 16 |
| Figure 5. Vertical subsurface flow CW. Source (Wang et al., 2017)..... | 18 |
| Figure 6. Perlite structure. Source: (The Perlite Institute, 2021) | 25 |
| Figure 7. The vertical subsurface flows CWs simulation in the Czech University of Life Science, Prague | 29 |
| Figure 8. Schematic diagram of experimental-scale CWs, represent two filling methods of substrate: reactor A: 150 mm gravel layer and 350 sand layers reactor B: 150 mm gravel layer, 300 mm perlite layer, and 50 mm sand layer. Created by the author (2021) | 30 |
| Figure 9. Schematic diagram of laboratory scale. Characteristics of reactors. Created by the author (2021) | 30 |
| Figure 10. The measurement of NH_4^+ by Agilent Technologies Cary 60 UV-Vis spectrophotometer | 34 |
| Figure 11. Average Length (g) of <i>G. maxima</i> Shoots and Roots..... | 37 |
| Figure 12. Average Weight (g) of <i>G. maxima</i> Shoots and Roots | 37 |
| Figure 13. Total Organic Carbon (TOC) concentration in the outflow ($\mu\text{g L}^{-1}$)..... | 39 |
| Figure 14. Total Carbon (TC) concentration in the outflow samples ($\mu\text{g L}^{-1}$)..... | 39 |

| | |
|---|----|
| Figure 15. Inorganic Carbon (IC) concentration in the outflow ($\mu\text{g L}^{-1}$) | 40 |
| Figure 16. The removal efficiency of IC, TC, and TOC (%)..... | 40 |
| Figure 17. Ammonium (NH_4^+) concentration in the outflow ($\mu\text{g L}^{-1}$) | 41 |
| Figure 18. Nitrate (NO_3^-) concentration in the outflow ($\mu\text{g L}^{-1}$)..... | 41 |
| Figure 19. Nitrite (NO_2^-) concentration in the outflow ($\mu\text{g L}^{-1}$)..... | 42 |
| Figure 20. Phosphate (PO_4^{3-}) concentration in the outflow ($\mu\text{g L}^{-1}$) | 43 |
| Figure 21. The removal efficiency of Ammonium (NH_4^+) and Phosphate (PO_4^{3-}) (%) | 43 |
| Figure 22. Ibuprofen in the outflow ($\mu\text{g L}^{-1}$) | 44 |
| Figure 23. Diclofenac concentration in the effluent ($\mu\text{g L}^{-1}$)..... | 44 |
| Figure 24. DCF metabolite: Diclofenac-4'-hydroxy in the effluent ($\mu\text{g L}^{-1}$)..... | 45 |
| Figure 25. IBU metabolite: Ibuprofen-2'-hydroxy in the effluent ($\mu\text{g L}^{-1}$)..... | 45 |
| Figure 26. The removal efficiency of IBU and DCF (%)..... | 46 |
| Figure 27. The content of Ibuprofen and Diclofenac and their metabolites in the plant roots ($\mu\text{g kg}^{-1}$)..... | 47 |

1. Introduction

Emerging pollutants have attracted increasing concern due to dramatic growth in population and rapid industrialization in the 20th century worldwide. Large-scale produce and use of EPs as goods, services, personal care products, pharmaceuticals, and further discharge of their processed products into the environment are some of the crucial causes of ecosystem disturbance in 21century. Moreover, water and air pollutions create a high load on the human immune system. Consequently, they have an extremely negative effect on all body such as increased morbidity and mortality and different kinds of allergies (Manisalidis et al., 2020).

Sources of EPs are agricultural, urban, and rural areas. The emerging contaminants, including personal care products, are known as (PPCPs), Non-steroidal anti-inflammatory drugs (NSAIDs), hormones, pesticides, plasticizers, industrial and household products, metals, food additives, solvents, flame retardants, and other organic compounds in the water generated mainly by human activities (WWW.UNESCO.ORG, 2019). For instance, PPCPs such as detergent for the washing machine may anticipate ensuring the daily human life in terms of comfort: time saver and alleviate manual labor. While on the other hand, their usage has an environmental harming by polluting and dwindling supply of resources and destructively altering the climate condition. Another significant negative contribution is that washing machines require high energy consumption from fossil fuels. Fossil fuels are burning for energy production, at the same time producing carbon dioxide and greenhouse gases. After the penetration into the air, they aggravate the global warming crisis. Furthermore, beyond the existing issues, the onset of the COVID-19 pandemic enhanced PPCPs production and medical consumables and caused an extremely high discharge of waste in the ecosystem. Hence, the consequences for the environment by releasing the debris, including non-biodegradable plastic syringes, pose additional challenges for ecologists.

Wastewater treatment plants (WWTPs) focus on removing contaminants from wastewater and, by effluent, discharge it into the water cycle. However, Corada-Fernández et al. (2017) have noted that most WWTPs were not designed to eliminate PPCPs, including NSAIDs. Therefore, in the world, especially in big cities, the overflow of the sewage system causes a significant content of EPs in the groundwater

and surface water. So, the occurrence of EPs in the environment their detection in different combinations is constantly reported and accounted for worldwide.

Once released into the environment, the degradation process begins. Unfortunately, the behaviour of many EPs is still insufficiently understood. Therefore it can be out of control and cause or aggravate undesirable consequences (Llamas et al., 2020). Furthermore, only a few commonly used contaminants, such as Ammonium N ($\text{NH}_4^+\text{-N}$), are toxicologically evaluated due to their constantly growing numbers (Tang et al., 2019). Therefore a rising interest over the last years in monitoring the presence and influence of EPs in the environment, mainly in surface water bodies (Corada-Fernández et al., 2017).

Constructed wetlands have been proposed and successfully used as an ecologically friendly option for wastewater treatment during the last decades. These alternative systems have been tremendously productive in preventing ecological impact and low operating costs (Fitch 2014). Therefore, CWs have been identified as a sustainable wastewater management solution worldwide. The components of CWs included: substrates, emergent/submerged vegetation, and water. The substrate plays a pivotal role in the adsorption within the pollutant's removal process. But even here, there are still many unexplored gaps because of a lack of familiarity and experience of this methodology and availability (Nelson et al., 2007).

2. Literature Research

2.1 Emerging pollutants

Demographic growth and economic activities lead to an expansion of anthropogenic contaminants in the environment (Richardson and Kimura 2020). As a result, many researchers extensively studied the multiply detrimental impact of humans on the surroundings due to their relevance. However, yet still many new threatening appears to both: ecology and human among emerging contaminants observed PPCPs, hormones, antibiotic resistance genes, by-products of drinking water, disinfection, UV filters, household products, metals, food additives, solvents, naphthenic acids, veterinary drugs, and various other compounds with unique physical and chemical properties which are products of human action discharged into the environment and harm to the ecosystem (Farré et al., 2008).

Emerging pollutants are a large, relatively new group of synthetic or naturally occurring chemical compounds that have not been studied before. They are not currently covered by existing water-quality regulations and are alleged by ecologists to be potential threats to environmental ecosystems, safety, and human health (Farré et al., 2008). The primary sources of EPs in the surrounding medium are WWTPs effluents and terrestrial run-offs, including an atmospheric deposition (Farré et al., 2008; Geissen et al., 2015).

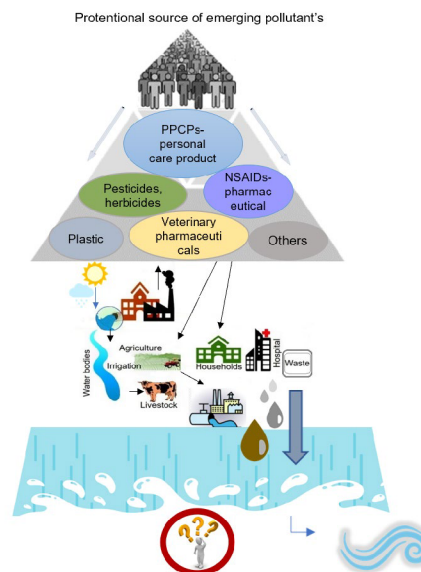


Figure 1. Source and pathways of EPs in the environment. Created by author, 2021

Since the very beginning of this century has been reported that every day, 2 Mio t agricultural sewage and industrial waste are discharged into the global water (IISD 2003) (Geissen et al., 2015), besides 75 to 80% of water pollution is caused by domestic sewage (Mehtab Haseena et al., 2017). According to the NORMAN network, in the water resources of Europe have been found and reported around 700 substances categorized into 20 classes (Geissen et al., 2015). Furthermore, analysis has shown that during the period between 1930 and 2000 covered by surveys and statistical the global production of manufactured chemical contaminants has risen from 1 million to 400 million tonnes each year (Gavrilescu et al., 2015). It was also highlighted by WWF (World Wide Fund For Nature, 2020) that still not enough information about the long-term effects on the ecosystem of some chemicals.

Moreover, the market has recently reached more than 3000 active pharmaceutical ingredients, including hormones, that create growing concern about the possible human health effects from long-term exposure to low-level concentrations of pharmaceuticals in the surface and drinking water (Richardson and Kimura 2020). For example, observation indicated that antidepressants have destructive effects on flora and fauna after being released into the ecosystem. The impact includes behavioural changes, morphological anomalies, fertility reduction of aquatic inhabitants, and general survivability of fish as a result as a whole (Sehonova et al., 2018; Reis et al., 2019).

The research was already making many attempts at the potential causal connection between human acts and the adverse health effects of EPs on the biota. During the last two decades, studies have reported the presence of EPs in the waterbody of Europe. Corada-Fernández et al.'s (2017) survey has provided a review of the existence of emerging organic groundwater contaminants (EGCs) found in the UK. Research in France focused on the effects of personal care compounds, including bactericide, antifungal agents, polycyclic musk's tonalite, and galactosidase, which is used in household products. Studies have shown widely detected all mentioned compounds in surface water and groundwater (Stuart et al., 2012). A study in Spain is dedicated to seeing PPCPs and disinfectants, among other products (Estévez et al., 2012). Data showed antibiotics and their degradation products as the most recognized compounds in the aquifer. But, alas, according to the Directive, only 33 PPCPs compounds have been included in the priority substances in surface water (DIR

2008/105 EC). The recent review on the sources of spread of EPs in European groundwater has identified pesticides, PPCPs, and pharmaceuticals as the most studied compounds (Corada-Fernández et al., 2017). The presence of EPs is widely detected in wastewater and groundwater in Poland. Based on the report by Kapelewska et al. (2018), in Poland, emerging compounds have been infiltrated in groundwater mainly from permeation from landfills. In the agricultural sector, the high fertilization indicators and pesticides have increased the discharge of nutrients and contaminants into the aquatic ecosystems (Matamoros et al., 2012). Due to farm business in some regions of the world, more than 50% of native freshwater fish species are at risk of extinction (Jean-Christophe Vié 2010; Geissen et al., 2015).

Moreover, the widespread of EPs and climate change affect the geographical spread of many dangerous infectious diseases (McMichael A. J., Confalonieri U., Brijnath 2012). Besides, pollution is costly. Welfare losses due to pollution water, air, and soil are estimated to amount to US\$4.6 trillion per year: which corresponds to 6-2% of global economic output (Landrigan et al., 2018).

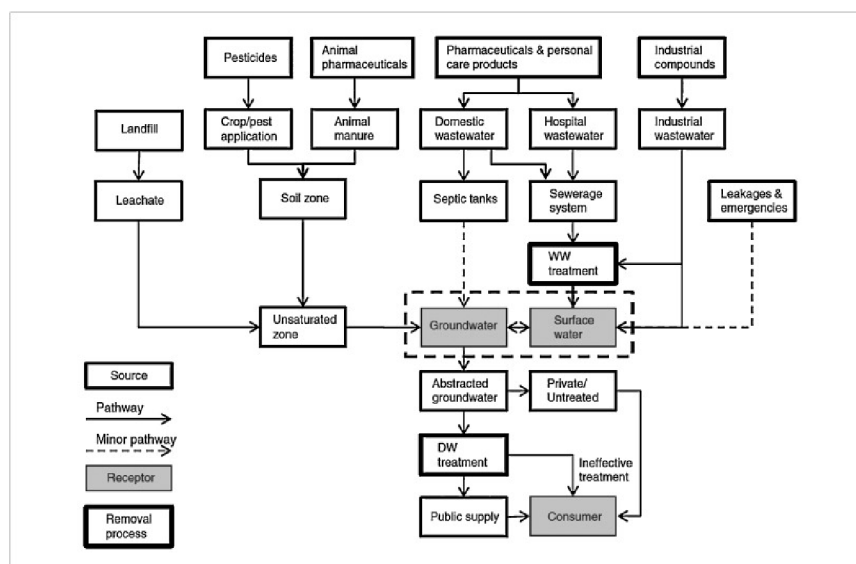


Figure 2. Schematic pathways of some EPs from sources to receptors, Copyright (Stuart et al., 2012)

A schematic view provided by Stuart et al. (2012), as shown in Figure 2, a route is groundwater-surface water interaction, where the effluent from sewage is discharged to surface water, and illustrate pathways for EPs, including PPCPs, urban and industrial contaminants to the groundwater (Gavrilescu et al., 2015). Unfortunately,

current state-of-the-art methods for sampling and analysis are still dedicated not for all but only to certain EPs classes (Geissen et al., 2015).

2.2 Ecotoxicity of EPs

The widespread industrial production and non-control of EPs in everyday life lead to increasingly detected many types of EPs in the environment even after the conventional water treatment process (Bell et al., 2011). Since water is a most valuable resource, the problems of sewage water treatment are today primordial from the perspective of pollution and further disturbance on environmental and ecological health (Nelson et al., 2007).

Since EPs are initially the product of human use, organic compounds enter the environment depending on their mode of usage and application. Therefore, EPs can be widely spread in the ecosystem once released through the WWTP effluents and terrestrial, including the atmospheric deposition pathway. For example, considering the WWTP cannot fully cope with pharmaceutical purification, the pharmaceuticals are released into aquatic systems after ingestion by humans and subsequent excretion in the form of the-metabolized parent compounds or through WWTPs (Farré et al., 2008). As a result, these compounds are released into the aquatic system and may be harmful to natural flora and fauna (Patel et al., 2019).

Some contaminants accumulate in food chains, can be highly toxic, and take different modifications depending on the emission environment. For instance, during the tests based on bacteria, algae, invertebrates, and fish for different compounds classes, antidepressants, antibacterial, cardiovascular, central nervous system pharmaceuticals, and antipsychotics were the more toxic (Stuart et al., 2012). Furthermore, it assessed the short-term acute toxicity of NSAIDs such as diclofenac, ibuprofen, naproxen, and acetylsalicylic acid towards algae and invertebrates. Based on its results, DCF is classified as a potentially harmful compound to aquatic organisms (Cleuvers 2004). The wide application of food additives such as Butylated hydroxyanisole and butylated hydroxytoluene prevents fat spoilage. To extend the “shelf life of food,” additives often include camphor, 1,8-cineole (eucalyptol), citral, citronellal, cis-3-hexanol, heliotropin, hexanoic acid, menthol, phenyl ethyl alcohol, triacetin, and terpineol. Some of these, for instance, can be implicated in endocrine disruptors (Stuart et al., 2012). Other studies have shown that nicotine has high toxicity

to humans' health compared to other alkaloids and neonicotinoid pesticides. The Whitehouse, Boullata, and McCauley (2008) overview provided the toxicity of artificial sweeteners as food additives. Thus was reviewed that their consumption has been indicated to cause mild to wide-ranging severe side effects from headaches to cancer. Up to now, the use of artificial sweeteners has remained controversial. Also, there has been increased interest in UV filters used for producing cosmetics and PPCPs. Due to the widespread use of sunscreens, the presence data appeared of UV-filters in a waterbody and their endocrine toxicity, particularly for the living organisms (Kunz and Fent 2006; Schmitt et al., 2008; Kolpin et al., 2002). These and many other combinations of pollutants produced and disposed of by humans may increase water resources content by viruses, bacteria, and fungi, which have antimicrobial-resistants to various classes of pathogens. Pharmaceuticals, including antibiotics, antifungals, and PPCPs, play a crucial role in speeding up this process. In other words, antibiotics contribute to the spread of antimicrobial resistance genes in the living environment (Frascaroli et al., 2021). Thus, emerging or re-emerging pathogens are the source of many waterborne diseases (Gavrilescu et al., 2015).

Recently, more often has attracted growing attention to littoral plastics debris (Tang et al., 2019). Due to their worldwide use in many human activities as single-use applications that cannot recover and recycle. It creates enormous problems for the habitat of water plankton, including ecosystems biodiversity loss and the decline in marine productivity (León et al., 2018). Significant effects of water pollution on human health include bacterial diseases caused by *Campylobacter jejuni*, *Shigella bacteria*, and many others. Also, hepatitis is a viral disease caused by contaminated water (Mehtab Haseena et al., 2017). There is a high current relevance between chronic effects of micropollutants. But, up to now, little information has been reported on their different outcomes and impacts (Farré et al., 2008).

A review by Fent, Weston, and Caminada (2006) has also reported a lack of information about the long-term effects of pharmaceuticals on aquatic organisms from a biological point of view. Despite significant successful researchers in analytical technology still a strong need to identify all potential pathways of influence of EPs on the environment (Boxall 2012). In 2005, was funded the NORMAN project which has been financially supported by the European Commission and has become helpful in

promoting and facilitating a wide-scope exchange of data on the occurrence and impacts of EPs in the world (Dulio et al., 2018).

2.2.1 Synergistic toxic effects

Most living organisms, including plants, undergo continuous stress by chemicals imposed by human activity. It has been reported that these contaminants can cause toxicity in aquatic species, animals, and critical organs of the human body. Up until now, the main focus was on the combined additive effects of chemicals that have a similar attitude. In contrast, the combined impact of different interacting substances has yet to be ignored or not studied thoroughly. The combined interactions or cooperation between two or more substances can significantly raise toxic exposure. However, the extent of different stressors that influence the ecosystem is highly controversial and has not been proven yet (Liess, Henz, and Shahid 2020). The studies of Pomati et al. (2008) and Pomati et al. (2006) explored the effects and interactions of a mixture of pharmaceuticals, including carbamazepine, IBU, and sulfamethoxazole, at low concentrations. They concluded that a combination of drugs at ng/L levels could inhibit cell proliferation, and waterborne pharmaceuticals may harm the aquatic system.

A few other reviews have demonstrated more widely disseminated synergistic interactions by comparison to additive and antagonistic effects. For example, research about synergistic results of marine pollution by Dulio et al. (2018) has reported that the magnitude of human impacts was higher in coastal zones than areas located more profound into the mainland. This conclusion is based on the probability of mutual influence of different pollutants from different media.

However, there has still been little information reported to date according to the synergistic toxicity of many EPs. Some compounds are still not fully understood (Farré et al., 2008). Moreover, the physicochemical properties of compounds, and the multidimensional nature of the environment, determine the unpredictable behaviour of the EPs in the ecosystem (Gavrilescu et al., 2015).

2.2.2 Removal efficiency in wastewater treatment plants

WWTPs are designed to remove contaminants and reduce pathogen load before releasing water into the environment (Ahmed et al., 2019). WWTPs usually

employ a primary, secondary, and tertiary water purification system (Patel et al., 2019). EPs in WWTPs are typically eliminated by photodegradation, biodegradation, sedimentation, plant uptake, and/or adsorption (Matamoros et al., 2012). However, up to now, WWTPs were never designed for the processing and disposal of pharmaceutical compounds. Indeed, many pharmaceuticals are environmentally persistent due to their specific design: they cannot be adsorbed effectively on activated sludge, and microbes used in secondary treatment cannot degrade them completely, and for the reason of their molecular structures, which resist oxidation, they continuously discharge into the ecosystem (Patel et al., 2019).

For the remediation, use physical, chemical, thermal, and biological methods. The physical treatment method contains adsorption, sedimentation, electro dialysis, evaporation, filtration, flocculation, reverse osmosis, and stream stripping. Chemical methods consist of ion exchange, neutralization, reduction, and precipitation. In turn, due to biological and chemical treatment, there is a chemical process of pharmaceuticals reaction into new metabolites, degradation products, or complete mineralization.

Nakada et al. (2008) have reported the effective removal of IBU, DCF, and other resistant pharmaceuticals. These results were achieved using a treatment process that included adsorption, photodegradation, and biodegradation. The previous researchers of elimination in WWTPs demonstrated a high variability of the data and the overall purification efficiency of some NSAIDs. Patel et al. (2019) indicated that remediation efficiencies of such pharmaceuticals as DCF in WWTPs can be less than 10%. But, on the other side, studies have found that IBU is a comparison well removed (between 60% to 99%) at most WWTPs (Smook, Zho, and Zytner, 2008). According to Yang Zhang et al. (2017), DCF show results in the degradation range from 20 to 40%, while IBU degrades faster due to aerobic conditions (>90% removal). Nevertheless, many pharmaceuticals remain in WWTP effluent and water bodies owing to their low removal efficiency and various harmful elements (Yamamoto et al., 2009). IBU is often detected in surface water (0-36.8 $\mu\text{g L}^{-1}$) and groundwater (0-3.1 $\mu\text{g L}^{-1}$) (Jia et al., 2021). A study in Brazil reported the occurrence of 28 drugs in high concentrations (up to 11.9 $\mu\text{g L}^{-1}$) in six drinking water treatment plants in Minas Gerais. Those compounds had varying removal efficiencies 32-100(%) in the drinking water treatment. The researchers in China during the study have found 54% of drugs

detected in rivers originated from untreated raw sewage, which is appropriate 50% of the total wastewater discharged into rivers (Richardson and Kimura 2020).

Below are examples that may affect the speed and quality of pharmaceutical removal. Sorption behaviour in the WWTPs is a complex purification process influenced by pharmaceutical and sludge characteristics. Electrostatic interactions and solution pH may play a significant role in protonated or deprotonated compounds in solution. Pharmaceutical and PPCPs persistence depend upon pH, sunlight, temperature, physicochemical characteristics, and the presence of different types of micro-organisms. Season fluctuation increases or slows down microbial biodegradation depending on solar activity and temperature. Furthermore, a high level of precipitation and runoff can cause WWTPs to overflow and lead to the release of undegraded pharmaceuticals (Patel et al., 2019). WWTPs can transform pharmaceutical metabolites into the parent drug before releasing it into the environment by deconjugation. The deconjugation of glucuronidated and sulfated DCF released active DCF and IBU produced from converting its hydroxy and carboxy derivatives (Patel et al., 2019). The process has been demonstrated with estradiol and 17 α -ethinylestradiol (D'Ascenzo et al., 2003). Although microbes play a central role in organics removal in WWTPs due to their contribution to the degrading process of many organic compounds such as biodegradation in the aquatic system, its performance generated numerous by-products. There is still little study of the effects of low doses and long-term exposure of pharmaceuticals on human health, and still not fully clear which key factors influence the removal process of the medications in the environment (Lopez et al., 2015).

Phosphorus (P) as a type of EPs likewise has been analyzed of effluent treatment work and was reported high-performance removal from wastewater. P is achieved by using the chemical precipitation process (Bali and Gueddari 2019). In contrast, the efficiency of removing nitrogen (N) compounds in various plants varied widely in time. They depended on the temperature of sewage and the ratio of BOD₅/TKN (Nourmohammadi et al., 2013).

In summarizing the data, the WWTPs issue is that it's one of the most expensive public industries in the EU in terms of energy requirements accounting for more than 1% of electricity consumption in Europe (Gandiglio et al., 2017). Besides,

the extensive use of chemicals also forced us to reconsider existing water purification methods (Patel et al., 2019). However, despite the proven positive effect of WWTPs on the mitigation of EPs from wastewaters, not enough attention has been paid to the fate of EPs in the natural aquatic ecosystem, restored, and CWs that were impacted by urban and agricultural run-off (Matamoros et al., 2012). Thus, currently, there is a sharp focus on removal, adsorption, and degradation of pharmaceuticals and other EPs, and seeking other advanced techniques less harmful and economical in terms of cost practices.

2.3 Pharmaceuticals in the environment: Ibuprofen and Diclofenac

At present, pharmaceuticals have occurred widely in the environment of industrialized countries (Tim aus der Beek et al., 2016). However, it is worth emphasizing that drugs are designed so that if used indiscriminately aimlessly, they create more likely harm to the health than benefits. Furthermore, large quantities of NSAIDs used to reduce moderate pain and inflammation, to treat rheumatic disorders such as DCF and IBU are sold as ‘over-the-counter.’

Urban domestic effluents are the primary and most extensive pathway of pharmaceutical contamination. Many pharmaceutically active compounds as anti-inflammatories and analgesics (IBU, paracetamol, DCF) have been identified in water since the first discovery in aquatic systems in the 1980s (Karen Bush 1997). The first nationwide USA study has detected 95 pharmaceuticals in 139 streams across 30 states during 1999-2000. In the early 2000s, more than 50 pharmaceuticals, EDCs, and illicit drugs were found in UK surface waters. The global expenditure on medicines for 2020 was \$ 1.4 trillion in 2020, which means about 30% more than in 2015. In 2013 the estimated overall yearly worldwide consumption of DCF for a human and veterinary use pharmaceutical drug was >1000 tons/year¹, and this amount increases every year (Memmert et al., 2013). North America dominates the DCF production. DCF Market size is expected to grow at a CAGR of 3.87% during 2020-2025. The increase of autoimmune and respiratory diseases and the increasing production of anti-inflammatory drugs are the key factors that stimulate the growth of the pharmaceutical market around the world (“Diclofenac Market Forecast (2020-2025)”, by Industry ARC) <https://www.industryarc.com/>.

Although somewhat impossible to calculate the actual world consumption of NSAIDs, Yongjun Zhang, Geißen, and Gal (2008) have estimated that about 940 tons of DCF are consumed globally based on an annual number of Intercontinental Marketing Services (IMS) health data. Along with leading and developed markets in the United States, emerging markets such as India, China, and Brazil also consume around N60 tons of DCF annually (the consumption estimation of DCF does not include veterinary use). In Australia, approximately 4 tons of DCF are used annually (Lonappan et al., 2016). Although their presence has been reported in China, India, Pakistan, Ghana, and others, the contamination in developing countries is still not explored enough (Patel et al., 2019). So researches in India have shown, DCF causes renal failure, which has led to high mortality of organisms 5-86(%) in Asian white-backed vulture adults (Oaks et al., 2004; Patel et al., 2019). A survey across 18 protected areas estimated that in 1991-1992 there were over 40 million vultures. However, between 1992 and 2007, three of India's vulture species declined from 97 to 99.9(%) due to cattle drug use which is toxic for the animals and birds (Shultz et al., 2004). The near-extinction of vulture has led to the spread of zoonotic diseases and increased the incidence of rabies ("Mongabay," n.d.).

Even though DCF has been included in the emergency medical list (EML) of 74 countries (Lonappan et al., 2016), India decided to be the first country to adopt regulations on the consumption of DCF. Due to its occurrence, The Vulture Conservation Foundation, The Royal Society for the Protection of Birds, BirdLife Europe, and then Vulture Specialist Group are campaigning for an EU-imposed, continent-wide ban on veterinary DCF (Lonappan et al., 2016). Apart from it, the United Kingdom has placed DCF on the list of "priority substances," which forced the water industries to remove DCF from wastewater (Mehinto, 2013). Furthermore, DCF is included in the previous Watch List of EU Decision 2015/495 since it is considered a "contaminant of emerging concern"(Lonappan et al., 2016; Yan Li et al., 2019).

After human medication, pharmaceuticals are excreted, intact or metabolized, via urine and faeces. The medicinal drugs enter the environment via flushing into drains, toilets, or disposal of household wastes. Then NSAIDs are deposited into municipal sewage systems (Eggen and Vogelsang 2015). Recently, in Southeast England, research has shown that 66% of the population have disposed of the excess pharmaceuticals into household wastes, and 12% flushed them down sinks and drains

(Figure 3) (Patel et al., 2019). The anti-inflammatory compounds IBU (up to 93 ng/l) and DCF (up to 261 ng/l) were amongst the most frequently detected in that area (Mehinto 2013).

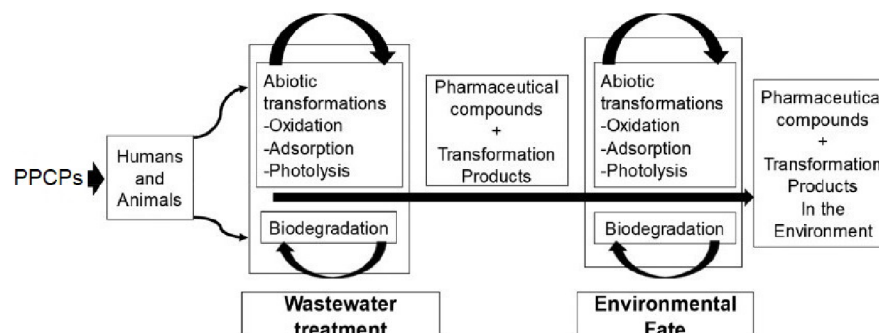


Figure 3. Transformation pathways of different pharmaceuticals in the environment. Copyright (Patel et al., 2019)

Since many pharmaceuticals are polar, they can be present in natural environments in unionized and ionized forms. After their release into the ecosystem, IBU, DCF, and many other medicines undergo photodegradation. It has been proved that they have indirect detrimental effects on several organisms at low and high concentrations via the food chain web. The medical products may also interact with similar types of receptors of organisms living in the environment (Patel et al., 2019). The author reported the toxic effects of microorganisms on the higher animals and studied the antibiotic resistance in bacteria and microbial communities. Additionally, attention was given to gene expression alterations, anomalous activities of protein and enzyme, and anomalies in the growth in rats, fish, and frogs (Patel et al., 2019; Kristiansson et al., 2011). Strong toxic pharmaceutical effects have been found in bacteria, *algae*, *Daphnia*, and others.

Almost 75% of the used DCF is present in surface water and infiltrate through the soil into groundwater (Sathishkumar et al., 2020). DCF has the highest acute toxicity among NSAIDs (Rosset et al., 2019). In addition, it has deadly effects by damaging renal and gastrointestinal tissue in several vertebrates, such as fishes (Mohebbi Derakhsh et al., 2020).

In the recent review (Sathishkumar et al. 2020), data was collected on the global occurrence of DCF in various water bodies, including aquatic animals. In addition, the author provides evidence of the presence of pharmaceuticals in effluents

from municipal wastewater treatment plants and in environmental compartments and biota. Some of them are presented below (Table 1).

*Table 1. The occurrence of DCF in various environmental compartments worldwide.
Copyright (Sathishkumar et al., 2020)*

| <i>Countries</i> | <i>DCF concentration</i> | <i>Nature of sample</i> | <i>Sampling point</i> |
|-----------------------|--------------------------------|-----------------------------|--|
| <i>Algiers</i> | <i>85 ng L⁻¹</i> | <i>Surface water</i> | <i>El-harrach Valley</i> |
| <i>Brazil</i> | <i>364 ng L⁻¹</i> | <i>Surface water</i> | <i>Jundiai River</i> |
| <i>China</i> | <i>121.6 ng L⁻¹</i> | <i>Surface water</i> | <i>Beiyun River Basin</i> |
| <i>Czech Republic</i> | <i>1.1 µg L⁻¹</i> | <i>Surface water</i> | <i>Elbe River Basin</i> |
| <i>Europe</i> | <i>72 ng L⁻¹</i> | <i>Surface water</i> | <i>Rivers from Spain, Belgium, Germany, and Slovenia</i> |
| <i>Germany</i> | <i>435 ng L⁻¹</i> | <i>Surface water</i> | <i>Lake Tegel and Havel River</i> |
| <i>Nigeria</i> | <i>57.16 µg L⁻¹</i> | <i>Surface water</i> | <i>Irrigation canal</i> |
| <i>France</i> | <i>2.5 ng L⁻¹</i> | <i>Groundwater</i> | <i>Wells of Herault Basin</i> |
| <i>Spain</i> | <i>380 ng L⁻¹</i> | <i>Groundwater</i> | <i>Underlying aquifers (urban)</i> |
| <i>Spain</i> | <i>25 ng L⁻¹</i> | <i>Drinking water</i> | <i>Mineral water in Valencia city</i> |
| <i>China</i> | <i>4.5 ng L⁻¹</i> | <i>Wastewater (inf/eff)</i> | <i>WWTPs in Chongqing</i> |
| <i>England</i> | <i>201 ng L⁻¹</i> | <i>Wastewater (eff)</i> | <i>WWTPs</i> |
| <i>Spain</i> | <i>15 ng⁻¹</i> | <i>Fish</i> | <i>Llobregat River</i> |

The study of Salvestrini et al. (2020) has also demonstrated data indicating the low removal efficiency of DCF in the conventional WWTPs, namely 10 ng L⁻¹-10 µg L⁻¹, with a peak value of 19 µg L⁻¹. Those data indicate DCF resilience to biological purification processes. Although DCF is one of the widely known and frequently used, research on the occurrence and toxicity of its metabolites in the environment is not well discovered (Lonappan et al., 2016). In contrast, IBU is one of the most studied organic micro-pollutants. It has high consumption and has been the third most sold pharmaceutical in Spain during the last decades (Ferrando-Climent et al., 2012).

However, there is still a lack of exploring about the presence and fate of its end product in the environment (Ferrando-Climent et al., 2012). Among several EPs in

several countries of the European Union at the exhaust of WWTPs and surface waters, IBP concentrations are higher than other analgesic and anti-inflammatory drugs (Jiménez-Silva et al., 2011; Stuart et al., 2012). Researches in Africa have shown that the quantity of IBU discharged into the Mbokodweni River by WWTP is higher than DCF (Amos Sibeko et al., 2019). According to the study, IBU and DCF can diffuse from river water into the roots of the water plant and maybe translocate into various parts of it. Experiment with *Eichhornia crassipes* has shown that plants (along with the substrate) can play a significant role in the uptake of pharmaceuticals from water, thus reducing water pollution (Amos Sibeko et al., 2019). Many other medications such as antidepressants Fluoxetine and Sertraline have been detected in surface water and wastewater effluent, at levels up to $0.54 \mu\text{g L}^{-1}$ and $0.93 \mu\text{g L}^{-1}$, respectively, for fluoxetine, and up to $0.08 \mu\text{g L}^{-1}$ and $0.09 \mu\text{g L}^{-1}$, respectively, for sertraline (Brooks et al., 2003; Metcalfe et al., 2010). Besides, Fluoxetine and its metabolite norfluoxetine have been found in fish tissues (Orem and Dolph 2002; Sehonova et al., 2018).

Based on the preceding, despite the pervasiveness of pharmaceuticals in water bodies, information of their effect on organisms has not yet been sufficiently reviewed (Kümmerer, 2009; Ferrando-Climent et al., 2012; Patel et al., 2019).

2.4 Constructed Wetlands

In recent decades constructed wetland systems have attracted much attention due to their high efficiency and capacity to remove many kinds of micro contaminants (Vasilachi et al., 2021). CWs are artificial engineered systems, serve sewage treatment processes that have low-tech, low maintenance, and use minimal energy requirements compared to conventional wastewater treatment plants. Therefore, it's affordable and has a strong potential for application in developing countries. Their ability can be provided as a WWTP's alternative (Patel et al., 2019). It is a system that evolves to respond to the climate's local influence and may promote an additional green zone for biodiversity and productivity. Besides, CWs prevent pollution and the degradation of natural ecosystems (Nelson et al., 2007). The CWs identified as a sustainable wastewater management option worldwide (Setyono 2016; Wang et al. 2011; INWRDAM, AMMAN 2016).

Predominantly the CWs, as an imitation of natural wetlands, aim to improve the quality of the water by performing as "living filters." The idea of recycling methods

is already relevant, and it will be essential to contribute enough fresh water in the coming decades (Green et al., 1997; Almutkar, Abed, and Scholz, 2018). The main aim of CWs to contribute to a second life for the water via its treatment with the minimum environmental impact. CWs provide considerable ecological benefits following the requirement of the EU framework directive (“European Commission,” 2000). Besides, the Water Framework Directive (WFD)(2000/60/EC 2000) provides a safety net, identification, monitoring, and a list of priority substances, and finally, the Environmental Quality Standards Directive (EQSD) (Directive 2008) (Geissen et al., 2015).

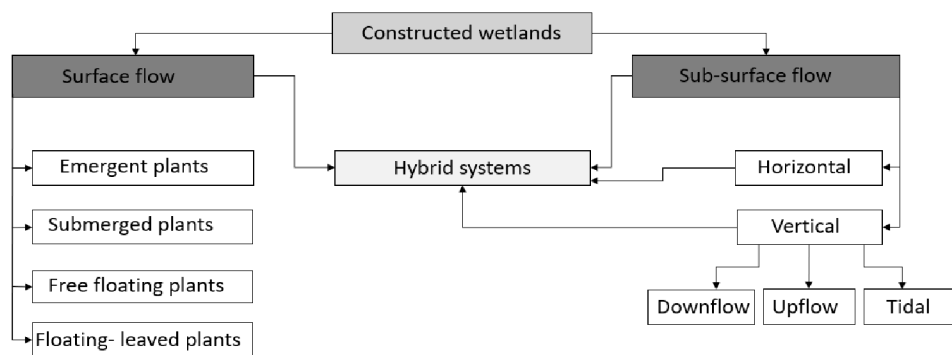


Figure 4. Types of CWs for wastewater treatment. Created by the author (2021)

The mechanism of CWs involves water, wetland vegetation, substrates, and the associated microbial assemblages, which help in treating wastewaters (Vymazal, 2010). The biological and chemical processes similar to WWTPs include sorption, sedimentation, hydrolysis, plant uptake, photodegradation, and microbial degradation. According to wetland hydrology, wetlands are classified as free water surface systems, subsurface, and floating treatment systems, according to the flow direction to the horizontal (H) and vertical flow (V). The different types of CWs may be combined in order to achieve a higher treatment water effect (Vymazal, 2010). A combination of different kinds of CWs known as Hybrid systems. Based on the various reviews covering CWs, the remediation of 115 pharmaceuticals was estimated a good result as the secondary treatment for eliminating the pharmaceuticals in all types of CWs: Vertical, Horizontal, and Hybrid (Patel et al., 2019).

In Europe are mostly uses VF wetlands operated for nitrogen removal using intermittent flow. Therefore, it becomes an alternative solution for efficiently treating

domestic wastewater (Perdana, Sutanto, and Prihatmo, 2018; Vymazal, 2020). HF subsurface flow and VF CWs are mainly used for the secondary and tertiary treatment of domestic and municipal wastewater (Vymazal 2010).

However, there is a dependence on removing microelements on climate conditions. Hence, the pollutant removal indicators vary considerably depending on season and region. For instance, low temperatures lead to limits in the removal of Nitrogen and Phosphorus (Wang et al., 2017). Due to results of recent research has been reported that Ammonium-Nitrogen ion ($\text{NH}_4^+\text{-N}$), total Nitrogen (TN), and total phosphorus (TP) removal were less efficient in winter than in summer, with a disparity ranging from 12.0% to 40.0%, 12.3% to 27.0%, and 6.1% to 34.0%, respectively (Yan and Xu, 2014).

Our study uses CWs with Vertical Subsurface Flow (VSSF CWs). Typically, Subsurface flow (SSF) wetlands consist of a layer of sediment, mostly of sand or gravel, (Figure 5) through which the polluted mass penetrates flows (Fitch, 2014). A VF CW comprises a flatbed of sand, water, microorganisms, and vegetation. The “interrupted dosing system” is when wastewater is inflow from the top of the system and then gradually permeates through the bed and is collected by a drainage network at the bottom. When the bed drains completely free, air can refill the bed with substrate again. The next dose of EPs traps this air and leads to good oxygen transfer and, thus, nitrifying. The beds with the VF of wastewater have favorable aerobic conditions to provide efficient nitrification and mineralization of organic matter (Debska et al., 2015). Thus, it makes VF CWs more efficient than other systems (Tsihrintzis, 2017).

Platzer's (1999) experience has shown that the interrupted dosing method has a potential oxygen transfer of 23 to 64 $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$. Therefore, the author concluded that oxygen diffusion from the air contributes much more efficiently than oxygen transfer through plants. In turn, Hans Brix (1997) revealed that the oxygen transfer through the plant has a potential oxygen transfer of 2 $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ to the root zone.

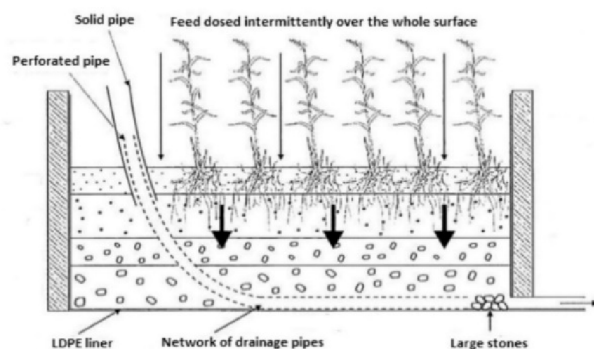


Figure 5. Vertical subsurface flow CW. Source (Wang et al., 2017)

The removal efficiency of VSSF CWs shows promising results in eliminating DCF, IBU, naproxen, and salicylic acid more efficiently compared to other types of CWs. The average removal rate of DCF and IBU is 20-50% and 50-70% removal efficiencies accordantly. Many studies show that more than 70% removal of paracetamol, caffeine, and sulpha drugs was achieved in VSSF CWs. However, there are still many gaps in research related to VSSF CWs used removing pharmaceuticals and the fate and stepwise removal pathways of the remediated compounds in the environment. Moreover, there are still not enough itemized descriptions of all involved elements in the degradation mechanisms mentioned above.

For this reason, CWs have been called “Black boxes” (Patel et al., 2019). Besides, the main operational problem of VF CWs is clogging (Stefanakis, Akratos, and Tsihrintzis., 2014). It also results from the incremental accumulation of organic and inorganic solids in the substrate, dense plant root development. This system feature leads to the poor performance of dewatering and pollutant removal, reducing aeration of the bed, nitrification, and disruption oxidation of organic matter (Tsihrintzis, 2017). Nevertheless, VF CWs are forward-looking technologies with many advantages and are predominantly appropriate for small settlements.

2.5 EPs removal Mechanisms in Constructed Wetlands

EPs removal is an essential target for CWs due to pathogens and metals in the water as significant health concerns for humans and aquifers (Norton, 2003). The EPs fate modeling needs to consider the catchment scale, the transfer of water, chemical particles between the substrate, type of the vegetations, groundwater, and surface water

(Geissen et al., 2015). CWs can effectively remove and convert many pollutants from different sources, including PPCPs, organic matter, nutrients, and pathogens. Organic matter removal is imperative due to decreased oxygen levels that lead to extra streams (Norton, 2003). The purification processes include sedimentation, filtration, UV light ionization, sorption. The activity of EPs depends on hydrolysis, biodegradation, temperature, redox potential, and many other factors (Llamas et al., 2020). Sorption is the most important chemical process, which includes adsorption and desorption operations (Montgomery, 2004). The physicochemical properties of EPs, such as pressure and polarity, determine their behaviour in the environment. Transformations of EPs depend upon many environmental conditions such as pH, temperature, and sunlight, which catalyze various degradation processes and may affect the final result (Patel et al., 2019). The biological approach is one of the most important mechanisms, and it includes photosynthesis, fermentation, respiration, nitrification, denitrification, and microbial phosphorus removal (Montgomery, 2004).

Conventional removal of N from water mainly includes a combination of aerobic nitrification and anaerobic denitrification and biological and physicochemical routes (C. G. Lee, Fletcher, and Sun, 2009). Nitrification is a chemoautotrophic process where the natural oxidation of ammonium converts to nitrate. It depends on temperature, pH value, ammonium-N concentrations, the water's alkalinity, inorganic C source, moisture, microbial concentration, and dissolved oxygen (Vymazal 2007; Lyu 2016). According to Vymazal (2007), Vertical flow CWs are very efficient in ammonia-N removal but very limited in denitrification. Thus, it was discovered the reduction of total nitrogen (TN) in VF CWs varied between limits 40 and 55%. Data indicate the removed load ranging between 250 and 630 g N m⁻² yr⁻¹ depending on CWs type and inflow loading.

2.5.1 Biodegradation process

“Biodegradation” is defined as decaying all organic materials by life forms. Since almost everything gets recycled in the microbiological sense, secondary metabolites or any degradation products from one organism can become the nutrient for others, decaying the remaining organic matter (Eskander and Saleh, 2017). Biological degradation can include bio-attenuation, bio-stimulation, and bio-augmentation processes (Ratnakar et al., 2016). Besides substrate loss, oxygen

consumption, and carbon dioxide formation (Bartz, 1998). The intensity of microbial transformation of chemical pollutants depends on the type and concentration of organic pollutants supply and the availability of Carbon, Nitrogen, Potassium, oxygen, optimum pH, and redox potential (Carberry and Wik, 2001). The biodegradation may be estimated by tests using conventional analytical methods like infrared spectroscopy, total organic carbon (TOC), dissolved organic carbon (DOC), and chemical oxygen demand (COD) (Bartz, 1998). Currently detected the increasing organic pollutants, their persistence, ability to bio-magnify, and bio-accumulate in the ecosystem (Ratnakar et al., 2016). The rising organic pollutants (synthetic and biogenic), their endurance, bio-magnification, bio-accumulation in the ecosystem, and the transformation of many nanomaterials in the soils lead to significant synergistic adverse effects on humans surrounding medium (Boxall, 2012). As a waste management technique, the bioremediation method uses bacteria, fungi, actinomycetes, earthworms, and green plants to remove or neutralize hazardous particles and reduce toxicity in polluted soil or water (Mazzeo et al., 2010; Aneyo et al., 2016). During the bio-stimulation process, decontamination of contaminated soil is due to the growth of microbes.

2.5.2 Phosphorus and Nitrogen removal in CWs

Nutrients, including nitrogen (N) and phosphorus (P), are essential for plant and animal growth and nourishment. N and P are primary nutrients. They are required in the most considerable amounts for the plants. At the same time, their overabundance in water causes many environmental issues such as eutrophication and soil erosion. They also can cause health effects for animals and humans (Mylavarapu, 2008). Furthermore, the excessive loadings of N and P are external factors that often reduce biodiversity and alter the plant community structure (Guignard et al., 2017). Moreover, P can accumulate in wetlands at a higher rate than N.

Phosphorus is widespread in agricultural fertilizers, organic wastes, sewage, and industrial effluent. The capacity of the wetlands to remove P is much lower and varied depending on many factors. Despite CWs being an effective method in removing N and P, the wetland sediments have a limited ability to adsorb P. Once saturated, CWs can no longer adsorb P; they may become a P source in the case of changing physicochemical conditions. Research in New Zealand estimated outlets of

farm drainage wetlands at Toenepi and Bog Burn and showed a much higher presence of P in the outflow over 3-5 years compared to the inflow. This study showed that wetlands were net sources of P (Ballantine and Tanner, 2010). For the removal of P from wastewater, the two most important physical processes are sedimentation of particulate P in the wetland and the adsorption of soluble P to the soil substratum. The overall P-removal capacity of CWs is highly dependent on the sorption characteristics of the substrate. Therefore the adsorption process of P to soil has been determined as the primary wastewater removal mechanism in the treatment wetlands. (Ballantine and Tanner, 2010). Within Batch incubation experiments performed by (Jamieson et al., 2002) measured the capacity of CWs of the dairy farm wetland in Pictou County, Nova Scotia, to remove P from the solution. The CWs in this area have been collecting wastewater since 1996. Non-linear regression analysis was used for the P adsorption characteristics based on the Langmuir adsorption model. It is the most frequently used P adsorption equation because it calculates theoretical P adsorption maxima. This study estimated and determined the P adsorption maxima in approximately 925 $\mu\text{g P kg}^{-1}$ for the deep zone soil, 924 $\mu\text{g P kg}^{-1}$ for the shallow zone soil, and 1600 $\mu\text{g P kg}^{-1}$ soil for the background soil (not receiving wastewater). As have shown, the P adsorption maxima for the deep zone and shallow zone soils don't offer a significant difference ($P > 0.05$). However, they were considerably lower ($P < 0.05$) than the background soil. The P removing through plants in the long-term elimination rate is an average of 50 $\mu\text{g/m}^2/\text{day}$ (Montgomery, 2004). Vymazal (2007) claimed that total phosphorus removal varied between 40 and 60% in all types of CWs.

Nitrogen is released into the environment mainly through agricultural processes since fertilizers especially contain nitrate, ammonia, ammonium, urea, and amines. In aerobic waters, nitrogen is generally present as N_2 and NO_3^- , and it may also be present as N_2O , NH_3 , NH_4^+ , HNO_2 , NO_2^- or HNO_3 , depending on the environmental conditions. A substantial amount of N is present in domestic wastewater, which generally contains no more than 3% nitrates and nitrites, but it mainly occurs as oxidized nitrite. Nitrogen may also be released into waterbody and soils from landfills (Lenntech, 2021). The N conversion of ammonia and ammonium to nitrate and nitrite is carried out by bacteria so-called nitrification process. A driver of the nitrification process is plants, algae, carbon, and oxygen (Montgomery, 2004). Even though N is essential for plants, they are relatively susceptible to NO_2 . Nitrates

themselves are not mainly considered toxic. But rising concentrations of N in surface layers lead to increasing plankton production and algal blooms, which may cause eutrophication. It has a negative impact, such as excess nutrients and fish deaths (Guignard et al., 2017). Nitrites are toxic to the human body in converting nitrate to nitrite, which may cause nausea and stomachache for adults. According to EPA standards, the maximum recommended concentration for nitrate is $10 \mu\text{g L}^{-1}$, the maximum level for nitrite is $1 \mu\text{g L}^{-1}$ (United States Environmental Protection Agency, 2021).

Removing N from the water WWTPs process often demands energy consumption and operation costs. The removal of N generally depends on environmental factors, including temperature, vegetation density, microbial type, climate, the distribution of wastewater, influent characteristics, oxygen, hydraulic residence time (HRT), etc. The changes within one factor can lead to changes inside the others (C. G. Lee, Fletcher, and Sun, 2009). The microbial metabolic pathway is a crucial N removal process, and as was noted in the research by L. Li et al. (2015), removing around 89-96% of the N.

The study by F. Li et al. (2014) used horizontal subsurface flow constructed wetlands (HSFCWs) and investigated the effects of dissolved oxygen (DO) and stage-delivery on N removal. In the research, the nitrification process was significantly enhanced by high DO concentration. Furthermore, the study by C. G. Lee, Fletcher, and Sun (2009) demonstrated the evidence of the role of artificial aeration. At the same time, the ammonia removal primary pathway in the free water surface (FWS) and subsurface-flow (SF) was the biological nitrification/denitrification process. A VFSS CW with 80% effluent recirculation demonstrates boosting the nitrification/denitrification reaction. The TN removal efficiency showed a satisfactory result of 72% (C. G. Lee, Fletcher, and Sun 2009).

Vegetation indicates high-efficiency removal of N and P. According to Hans Brix's (2003) research, up to 50% of N input was absorbed by plants. Based on the report of C. G. Lee, Fletcher, and Sun (2009) denitrification, the process in the CWs may remove 60-70% of the total removal nitrogen, and 20-30% of that amount is derived from plant uptake. Koottatep and Polprasert's (1997) research showed the range of N removal efficiencies of the CWs between the limit of 20-90%. Within the

process of taking ammonia and nitrate by macrophytes, the inorganic N forms convert into organic compounds and serve as a building material for cells and tissues (C. G. Lee, Fletcher, and Sun, 2009). The main factors that supported the growth rates of macrophytes and bacteria are planting depth, temperature, pH, and dissolved oxygen concentration (C. G. Lee, Fletcher, and Sun, 2009).

2.5.3 Sorption: an overview. Role of the substrate in the sorption process

The use of solids for removing pollutants from liquid solutions has been widely used over the centuries. The sorption principle involves separating a substance from one phase (liquid) by its accumulation at the surface (substrate). It is characterized by surface area pore volume, porosity, and polarity (Y Zhang et al., 2017). Sorption to solids is a significant way to remove EPs from an aquatic ecosystem (Patel et al., 2019). The sorption is strongly dependent on the features of the substrates (Boxall, 2012). There are two types of sorption processes: adsorption and desorption. The adsorption process is performed by methods of weak van der Waals interactions, hydrogen bonding, electrostatic attractions, charge-transfer complexes, strong chemical bonds, and electron transfers. The solid surface's sorbent properties and solution chemistry considerably influence the sorption of pollutants in soils (Yu Zhang, 2016). In our study estimated and compared the adsorption capacity of the substrate.

Low cost, low adsorbate concentrations, the possibility of long-term processing, reusing, and recycling of adsorbents are the main advantages of adsorption processing for water purification. Besides, adsorption can remove a wide range of organic and inorganic compounds and eventually release fewer toxic products than another conventional treatment method (Patel et al., 2019). Due to the broad structural diversity of pollutants understanding the different sorption and degradation pathway of EPs in soils and water are determining factors for predicting their mobility, leaching to groundwater, and as a result, the impact on the environment (Corada-Fernández et al., 2017). Various contaminants enter into the soil or aquatic ecosystems in the form of a different complex of compounds.

The sorption capacity of pollutants can vary significantly in different soil types. Therefore, various sorbent materials are widely used for water treatment works.

Among them is the sand, biochar, perlite, activated carbons, zeolite, clays, cotton fibers, etc. For example, the recent study in subtropical climate using emergent vegetation in combination with a porous-local ground volcanic rock as a filter medium showed that taking into account the adsorption process was 62.5% and 59% of removal efficiency of pharmaceuticals (Tejeda, Barrera, and Zurita, 2017). However, there is still minimal information on NSAIDs. Therefore, there is a strong need for scientific research on the migration and sorption of different NSAIDs, such as naproxen, DCF, and IBU (Yu Zhang, 2016).

The two substrates, sand, and perlite, which are widely applied, have been used in this research. Sand is a granular material with medium size 0.5-0.25 mm and various compositions containing finely divided rock and mineral particles. Sand medium is an effective removal mechanism for high P-sorption capacity; it shows high removal rates of nitrate, nitrite, and different organic contaminants from contaminated groundwater in subsurface flow CWs. Substratum supports plant growth and the attachment of microbial biofilm in the CWs (Koottatep and Polprasert, 1997). The sorption ability of sand depends on its physicochemical characteristics. The research results of Xu et al. (2006), based on the experiment of 9 substrates' P sorption efficiency, have found that sand sorption efficiency varied from 130 $\mu\text{g L}^{-1}$ to 290 $\mu\text{g L}^{-1}$, and also the P-removal features of sands of different geographical origins changed significantly. However, H. Brix et al. (2001) experiments have reported that sand is an ineffective applicant for long-term P treatment. On the other hand, it has solid hydraulic permeability and P dissolution capability. Despite sands on their own having relatively low P-sorption capacities, but due to mixing with other substances, their P absorbency and retention features may increase (Ballantine and Tanner, 2010). The study of Westerhoff et al. (2018) in the metropolitan area of Minneapolis-St. Paul, Minnesota, examined the occurrence and removal of 384 emerging contaminants in three large stormwater pipes and three pairs of iron-enhanced sand filters. The research results have shown a total of 31 contaminants identified in $\geq 50\%$ of the samples. Furthermore, the study demonstrated a high seasonal and site-type dependence for several EPs. Additionally, it has been revealed that iron-embedded sand filters significantly removed 14 of the 48 most detected contaminants with average removal efficiencies of 28-100%. In New Zealand, various experiments indicated that sands adsorbed and retained very few amounts of the sorbent P. At the same time was admitted that sorption characteristics

were highly variable between the type of sands, their structure, size of particles sands. It also has been found that masonry sand had a P-sorption capacity of only 0.058 g P kg⁻¹, and it easily desorbed P in more dilute solutions (Ballantine and Tanner, 2010).

Since fluvial sands have low chemical activity, they can be used as efficient filter materials and applied as alternate substrates in CWs (H. Brix, Arias, and Del Bubba, 2001). Furthermore, CWs with vertical flow operated systems are less liable to clogging (due to intermittent loading, which leads to alternating oxic and anoxic phases) than horizontal flow subsurface systems. Therefore VFCWs, since the end of the 20th century, became more popular in Europe. On the other hand, the commonly used fluvial sand filters have a relatively short life span due to exceeded loading rates. Since typically treatment systems need and $\leq 80 \text{ mm d}^{-1}$ loading rates there are very often not sufficient for urban wastewater treatment. Studies by Machate et al. (1999) have reported high nitrate and nitrite removal rates from contaminated groundwater in lava sand filtered CWs. Within this research, the adsorption of polycyclic aromatic hydrocarbons has been estimated. It shows higher adsorption rates onto the lava substratum of the wetland system. However, there is little study on lava sands as filter materials in CWs (Bruch et al., 2014).

Perlite as a sorbent shows environmentally friendly behaviour. It is an amorphous volcanic glass that usually contains between 2% and 6% water. It is created by the hydration of obsidian. It has a low density (lower than water) and high porosity, which increases its adsorption capacity and provides water and air holding (Figure 6). Perlite has a neutral pH and contains no chemicals or nutrients, and it also may overcome the problems related to the large, hazardous sludge production (Petrella et al., 2018). Perlite particles represent an aggregate of microscopic pathways that can filter and purify water, liquids, and pharmaceutical products (The Perlite Institute, 2021). Due to the high carbon cost of its production and regeneration, perlite may be considered an alternative to conventional activated carbon for adsorption purposes.

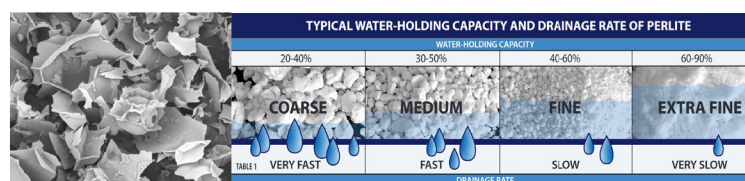


Figure 6. Perlite structure. Source: (The Perlite Institute, 2021)

The scientific study by Dordio et al. (2007) has recently reported the ability of expanded perlite to promote the removal of phenoxy acetic acid in CWs by sorption. Moreover, perlite has shown positive results as a sorbent for diuron, copper, 3,4-dichloroaniline, and glyphosate from model liquid matrices (Huguenot et al., 2010). Khudr et al. (2021) found a positive synergistic adsorption effect on treating copper pollution of water by adding iron to perlite. The collaboration effect of the iron and perlite indicates considerably functional in terms of rising the speed of copper uptake in a short time. Additionally, the experiment had concluded that it could further reduce the pressure on the environment. Within the study of Yifei Li et al. (2014), the adsorption characteristics of perlite and sand were compared with other absorbents, such as light expanded clay aggregate (LECA). The LECA possessed a high sorption capacity for the removal of clofibric acid in comparison to expanded perlite. On the other hand, the perlite showed a minimal sorption capacity, and the sand did not show any sorption capacity at all (Yifei Li et al., 2014). Although the perlite is a widely used material nevertheless, up to now, it has almost not been evaluated as an adsorbent for pharmaceutical removal (Bastani et al., 2006; Tejeda, Barrera, and Zurita, 2017).

2.5.4 Importance of plants in CWs

The successful operations of the wetland system are dependent on the interactions between plants, substrates, wastewater characteristics, microorganisms, and operational settings (Calheiros et al., 2009). Plants play essential roles in removing contaminants in water and soil (J. H. Lee, 2013). According to the life form, the macrophytes growing in wetlands may be classified into floating-leaved aquatic macrophytes, submerged aquatic macrophytes, and emergent aquatic macrophytes (Hans Brix, 2003). The main plant's roles are the transportation of oxygen to the root zone, antimicrobial compounds production, provision of substrate for attached bacteria growth and its activities, removal process, and nutrient uptake (Setyono, 2016). The uptake and storage rate of nutrients by plants depends on the quantities and concentration of the nutrients in the tissues (C. G. Lee, Fletcher, and Sun, 2009). Root surfaces have extensive surface areas and high-affinity chemical receptors. Therefore, it is essential for adsorption since root surfaces bind pollutants and nutrients. In soils, the adsorption processes are less efficient than the liquid medium due to competition between roots surfaces with various particulate soil materials (Meagher, 2000). The

rhizosphere is the most extreme ecological habitat in the soil. It is a narrow area around the plant roots in the ground under the direct influence of microorganisms of the higher plants (Bhosle, 2013; Maheshwari, Aeron, and Saraf, 2013). Bacteria can form close associations with roots, and their growth is closely linked to the metabolic activity of the plants involved in the process. Organisms provide a plant with a compound or nutrient such as N, P, or Fe. Their influences depend mainly on soil composition. The concentrations of nutrients in plants are highest at the beginning of the growing season and decrease gradually as the plants mature (Bhosle 2013).

Various experiments showed macrophytes' significant and positive effect on pollutant removal in the CWs (Brisson and Chazarenc 2009). Previous studies have shown that plants enhance treatment efficiency by contributing to the settling of particulates. They adsorb solutes and take up inorganic and organic compounds (Wang et al., 2017). Additionally, the planted CWs show higher pesticide and pharmaceuticals removal rates than wetlands without vegetation (Lyu 2016). According to Vymazal (2020), vegetation plays a commendable role in removing some medications. Among them are DCF, IBU, amoxicillin, ampicillin, carbamazepine, caffeine, erythromycin, naproxen, ketoprofen, salicylic acid, sulfadiazine, sulfamethazine, and sulfamethoxazole. Several plant species such as *Phragmites australis*, *Typha angustifolia*, and *Typha latifolia* have been highlighted for use in pharmaceutical wastewater treatment wetlands. However, knowledge is scarce about the toxic stress to plants caused by pharmaceuticals (Yifei Li et al., 2014). Debska et al. (2015) mentioned in a study that in recent years in Poland, there have started to be adopted to use the CWs with an aquatic, perennial grass *G. maxima*. This research will be used Emergent aquatic macrophytes, *G. maxima*, as a wetland plant.

3. Objectives

Large-scale production and consumption create a heavy load on conventional WWTPs, which are not designed for many pollutants, including medicals that appear in large amounts in the water. Additionally, the COVID-19 pandemic raised the release of a considerable amount of medicine in the sewage system. Thus, an urgent need arose to use CWs in terms of efficiency, economy, and ecologically friendly application.

As noted above, NSAIDs' removal method in CWs, including IBU and DCF and sorption efficiency of given absorbent, are still not thoroughly studied. Their further fate is still not well understood. Therefore the primary purpose of this research was:

-To investigate and compare the adsorption behaviour of the chosen substrate: sand and perlite for selected EPs in VSSF CWs.

-To analyze and estimate their role and purification ability in CWs for removing EPs, including pharmaceuticals IBU and DCF.

The research may provide data and knowledge about the purification process. In addition, the obtained data can be helpful at improving methods of elimination of EPs in CWs and mitigating their impact on aquatic and human life. Conclusively, the study can shed light on issues related to the impact of EPs on the ecosystem to contribute to future researchers.

Initially had planned to study both the adsorption and desorption capacity of sand and perlite. Unfortunately, due to the unpredictable situation of the COVID-19 epidemic in the Czech Republic, the desorption capacity of given substrates couldn't be achieved.

4. Method and materials

The experimental section of the thesis is based on the small-scale experimental sub-surface vertical constructed wetlands, which were established and used on the Czech University of Life Sciences campus in Prague.

The CW's setup comprises the innovative KG-system (PVC) pipes, substrates (sand, gravel, and perlite), wetland plants, and water outlets. The observed device consisted of 6 reactors grouped into two categories (A, B) to simulate the VSSF CWs. According to the root characteristics, a common wetland plant species, *G. maxima*, were selected. The plants have been acquired from a pond located on the Czech University of Life Sciences campus.



Figure 7. The vertical subsurface flows CWs simulation in the Czech University of Life Science, Prague

Sand samples were collected, air-dried, and then sieved to a particle size < 2 mm. Before cultivation, to remove soil particles, the roots of each seeding were washed with tap water. The testing system has required the construction of a special coating that may protect the reactors from external environmental impacts such as the ingress of rainwater. To prevent infiltration of water inside the system was installed in the form of a translucent polyethylene canopy set over the entire area of the experimental testing device. The measures were necessary to avoid flooding or the penetration of the outside water into the lower part of the columns and for the convenience of taking

outflow. Each experimental pipe system has been installed on a metal frame that raised the whole system to a level of approximately 50 cm from the ground level (Figure 7).

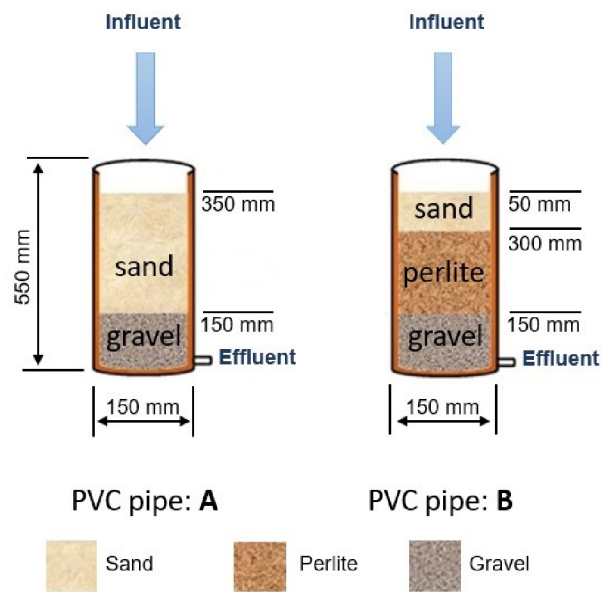


Figure 8. Schematic diagram of experimental-scale CWs, represent two filling methods of substrate: reactor A: 150 mm gravel layer and 350 sand layers reactor B: 150 mm gravel layer, 300 mm perlite layer, and 50 mm sand layer. Created by the author (2021)

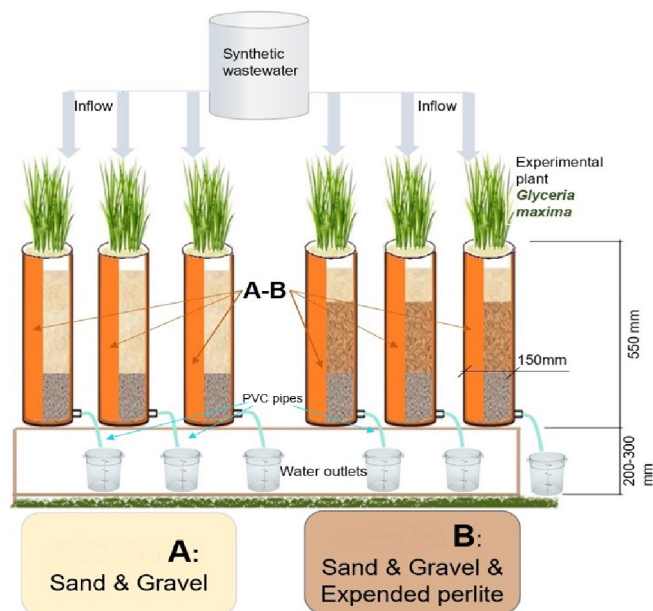


Figure 9. Schematic diagram of laboratory scale. Characteristics of reactors. Created by the author (2021)

Each column of the physical models had an open-top imitating natural water bog. All the reactors had a sampling point in the form of an orifice with a hose for the

water running down, located 25 cm above the plastic bottom. Each of the six reactors has dimensions 150 mm in width and 550 mm high and consists of a plastic base and column which made watering (Figure 8). All the six individual systems of CWs were carried out in the PVC materials column, divided into three layers: bottom layer, middle layer, and top layer for the type and number of substrate layers.

Category A reactor contained gravel and sand-based substrate. The A method of the substrate consisted of two layers: a 150 mm gravel substrate matrix (bottom layer) and a 350 mm sand matrix (top layer). In turn, category B reactors included gravel/sand and perlite. The B method of the substrate comprised three layers: with a 150 mm gravel substrate matrix (bottom layer), 300 mm expanded perlite matrix (middle layer), and 50 mm of the sand matrix (top layer). The study has been operated continuously for five-month from July to November 2020.

4.1 Experimental setup

The treatment was divided into three replication phases to evaluate the adsorption of two different substrates.

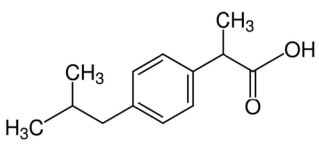
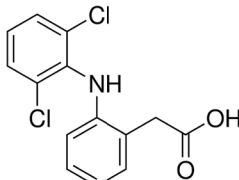
First of all, the solution of EPs was prepared as an inlet included IBU and DCF. It was designed to simulate municipal sewage. The composition of the nutrient solution is detailed in Table 2. IBU of high purity grade and DCF sodium salt (> 98%) were purchased from Sigma-Aldrich. Their physicochemical capacities are given in Table 3. After that, CWs were established and a solution prepared.

The first phase of treatment was started. Within this stage, the hydraulic loading has been intermitted of 2 L / 4 d (the water was kept in the PVC pipes for two hours and then drained). The replications were implemented during the whole five-month study period. Also, each column was irrigated with 10% strength synthetic wastewater in the first two-month to avoid excessive nutrients.

Table 2. The concentration of nutrients in wastewater solution prepared to simulate municipal sewage (mg L^{-1})

| <i>Reagent</i> | <i>Concentration, mg L^{-1}</i> | <i>Microelements</i> | <i>Concentration, mg L^{-1}</i> |
|--|---|---|---|
| <i>Urea</i> | 104 | <i>CuSO₄·5H₂O</i> | 0.01 |
| <i>NH₄Cl</i> | 16 | <i>FeSO₄·7H₂O</i> | 0.45 |
| <i>CH₃COONa·3H₂O</i> | 255 | <i>MnSO₄·H₂O</i> | 0.02 |
| <i>Peptone</i> | 20 | <i>Pb (NO₃)₂</i> | 0.02 |
| <i>KH₂PO₄</i> | 41 | <i>H₃BO₃</i> | 0.04 |
| <i>Yeast extract</i> | 132 | <i>Na₂MoO₄·2H₂O</i> | 0.02 |
| <i>Skim milk</i> | 59 | <i>KCr (SO₄)₂· 12H₂O</i> | 0.02 |
| <i>NaHCO₃</i> | 25 | - | - |
| <i>MgSO₄·7H₂O</i> | 41 | - | - |
| <i>CaCl₂·6H₂O</i> | 28 | - | - |

Table 3. Physical-chemical capacities of pharmaceuticals. Source <https://pubchem.ncbi.nlm.nih.gov/>

| <i>Pharmaceuticals</i> | <i>Structure</i> | <i>Molecular Weight, g/mol</i> | <i>Solubility in water, mg L^{-1}</i> |
|-------------------------|---|--------------------------------|---|
| <i>Ibuprofen (IBU)</i> |  | 206.30 | 21 |
| <i>Diclofenac (DCF)</i> |  | 296.15 | 4.82 |

During the second phase of treatment in the third month, the systems were run with 100% synthetic strength wastewater.

After that, the third step began. Since the beginning of the third month, IBU and DCF in a volume ($500 \mu\text{g L}^{-1}$) and ($100 \mu\text{g L}^{-1}$) respectively were mixed with 100% synthetic strength wastewater and added into each CW system until the end of the study. The added medicals have had their concentrations close to their levels in the influence of sewage water. All three experimental stages are given in Table 4.

Table 4. The replication phases during the five-month study period

| <i>Study period, months</i> | <i>1-st month</i> | <i>2-nd month</i> | <i>3-d month</i> | <i>4th month</i> | <i>5th month</i> |
|------------------------------------|---|--------------------------|---|-------------------------|-------------------------|
| <i>1 phase</i> | <i>hydraulic loading intermitting of 2 L / 4 d</i> | | | | |
| | <i>irrigation with 10% synthetic strength wastewater</i> | | | | |
| <i>2 phase</i> | | | <i>100% synthetic strength wastewater</i> | | |
| <i>3 phase</i> | | | <i>IBU ($500 \mu\text{g L}^{-1}$) and DCF ($100 \mu\text{g L}^{-1}$) mixed with 100% synthetic strength wastewater and added into each CW system</i> | | |

4.2 Sample analysis

4.2.1 Water quality parameters

The experimental sorption tests were conducted for each selected EPs in the soil following OECD Guideline No. 106 (OECD 2000). In order to estimate the substrate sorption capacity, the water quality analysis included a few steps. Water was collected in a plastic bucket, and then the volume was measured. The water outflow was collected at the 25 cm depth sampling point at each of the six reactors. Then was filled in special containers separately for each collector with 0.5 L and transported to be measured in laboratories in the university.

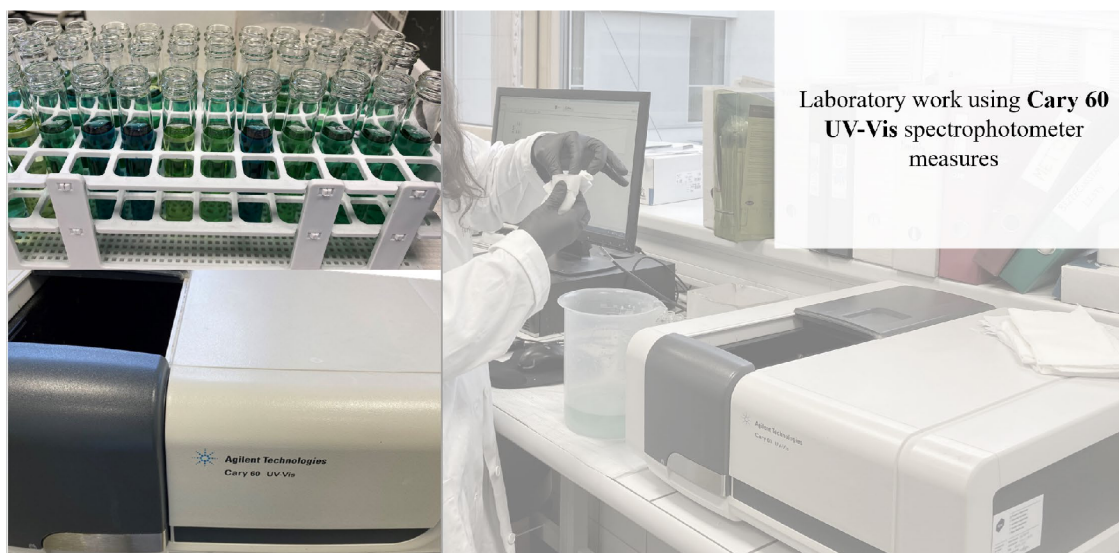


Figure 10. The measurement of NH_4^+ by Agilent Technologies Cary 60 UV-Vis spectrophotometer

All experiments were done at ambient laboratory temperature and a constant temperature between 20 °C and 25 °C. Outflow samples in the six CWs were taken every eight days and were analyzed. At the first stage, the data were collected for the following quality rates. The measurement of the TN and TOC were taken in the laboratory using the Primacs ^{SERIES} TOC analyzer (Skalar, Dutch). PO_4^{3-} , NO_2^- and NO_3^- were analyzed by 883 Basic IC plus (Metrohm, Switzerland). The measurement of NH_4^+ was undertaken by Agilent Technologies Cary 60 UV-Vis spectrophotometer. The indophenol method using Agilent Technologies Cary 60 UV-Vis spectrophotometer was carried out for NH_4^+ . For this purpose, alkaline and dyeing agent solutions have been prepared (Figure 10).

The method of preparing the alkaline solution was to dissolve a sodium hydroxide (NaOH) in deionized water and add Sodium Dichloroisocyanurate Dihydrate ($\text{C}_3\text{N}_3\text{O}_3\text{CL}_2\text{Na}_2\text{H}_2\text{O}$) in the following appropriate period to reach room temperature (around 20-22 °C). Each solution was added in quantities specially calculated for this purpose. In turn, the preparation of the coloring agent consists of adding into a deionized water two chemical compounds: sodium salicylate ($\text{C}_7\text{H}_5\text{O}_3\text{Na}$) and sodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7\cdot 2\text{H}_2\text{O}$) in calculated volumes. Then the dissolution of sodium nitroprusside dihydrate $\text{Na}_2[\text{Fe}(\text{CN})_5(\text{NO})]\cdot 2\text{H}_2\text{O}$ was added to the samples for the dissolving. Both were placed in a dark container to be protected from lighting and stored in the refrigerator. Subsequently, each solution was

pipetted into a reaction tube and left for 1 hour. Finally, each sample was measured using a spectrophotometer (655 nm wavelength with 1 cm corvette).

The measurement of the TN and TOC begins with placing samples on a rotating disc established in the high-temperature reactor (750-950 °C). Then by air transferring the converted particles to the particular detectors, their concentration is measured. Inorganic carbon dependent on atmospheric pressure was measured by the acidification of the sample, which provides the equilibria to CO₂. TOC detection is an essential measurement due to its influence on human health. The TOC calculated from the equation: $TOC=TC - IC$

4.2.2 Analysis of IBU and DCF and their metabolites in CWs

The aboveground plant piece has been taken every 16 days. The IBU and DCF content in the plant root and rhizosphere was measured. The plants were harvested at the end of the experience.

Then the root length and shoot height were measured. The rhizosphere substrate was collected and immediately at the same time with fresh plants were placed in a 40 oven for 120 h to prepare a dry sample. The dry substrate should have been used to assess its adsorption capacity of EPs performed by equilibrium isotherm experiments but didn't finish it because of the COVID-19 pandemic. The results should have demonstrated the adsorption capability of two different substrates at the soil-water interface at a specified temperature. The content of the IBU and DCF and their metabolites, such as 2-hydroxy ibuprofen (2-OH IBU) and 4'-hydroxy diclofenac (4'OH DCF) in plant tissues included roots and shoots, and rhizosphere substrates were analyzed every 12 days in the laboratory by liquid chromatography-mass spectrometry (LC-MS). Additionally, the removal efficiency of IBU and DCF (%) was investigated. The measurement stages for chemical compounds and indicators of water quality are represented in Table 5.

After the end of the experiment, which was done at the end of November 2020, all of the substrates, plants, roots, and shoot samples were measured and stored carefully for future analysis.

Table 5. The measurement stages for chemical compound and indicator of water quality

| The measurement stages | Chemical compound and indicator of water quality |
|---|--|
| 1. Water quality parameters ($\mu\text{g L}^{-1}$) | (TC) total carbon (TOC) total organic carbon (IC) Inorganic Carbon (PO_4^{3-}) phosphate (NO_2^-) nitrite (NO_3^-) nitrate (NH_4^+) ammonium |
| 2. Plant analysis | root length (cm) shoot height (cm) root and shoots weight (g) |
| 3. Analysis of metabolites of the IBU and DCF in plant tissues, roots, shoots, rhizosphere substrates | 2-hydroxy ibuprofen (2-OH IBU), $\mu\text{g/kg}$ 4'-hydroxy diclofenac (4'OH DCF), $\mu\text{g/kg}$ |

5 Results

The experiments lasted for five months, beginning in July and concluding in November, have been completed successfully. Unfortunately, my study did not cover the desorption study process for substrates to the organic compounds (IBU and DCF) as planned earlier due to the situation of the COVID-19 epidemic in the Czech Republic. Nevertheless, data representation has been provided based on the initially established research interests, including definition and comparison sorption capacity of two different substrates, sand and perlite, plant measurement and data, estimation contents of EPs, pharmaceuticals, and their metabolites.

5.1 Plant biomass

The five-month study has shown the results and demonstrated the influence of the biomass of the planted vegetation *G. maxima*. The measurement included the length (cm) of shoots and roots and weight shoot and root weight (g), respectively. The

biomass using sand as a filter had a root length of 20.33 cm and shoots length of 33.67 cm for a total of 53.97 cm. In turn, using perlite has a comparatively prevailing effect on average plant biomass with a root length of 24.33 cm and shoots length of 43.00 cm for a total of 67.33 cm (Figure 11).

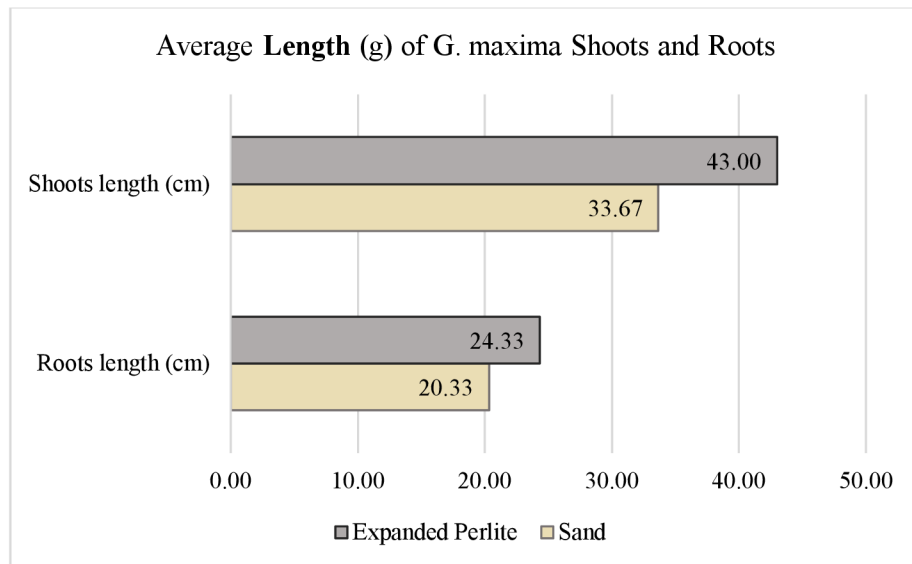


Figure 11. Average Length (g) of *G. maxima* Shoots and Roots

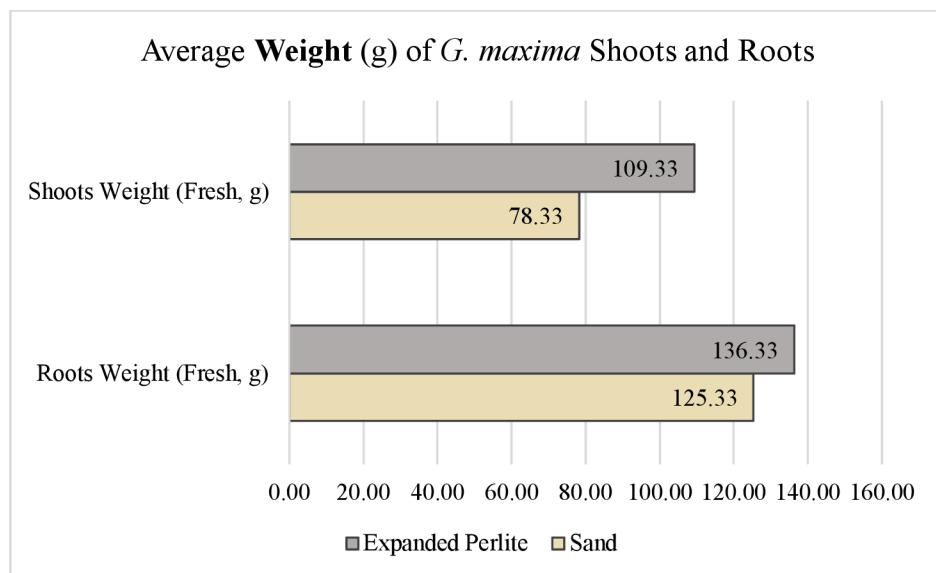


Figure 12. Average Weight (g) of *G. maxima* Shoots and Roots

Because of the overall volume, the average weight of plants in perlite as a substrate was also significantly higher, with root weight 136.33 g and shoots weight of 109.33 g with a total weight of 245.66 g (Figure 12). On the other hand, data for the plants for substrate sand showed weaker fasting results. As a result, the average weight

of plants in the sand as a substrate was significantly less, with root weight 125.33 g and shoots weight of 78.33 g with a total weight of 203.66 g.

These indicators may be explained by the characteristics of the structure of substrates. Sand being less porous than perlite, has a lower ability to retain a liquid and nutrients and consequently lower absorption capability. The perlite has high porosity, and that's why it can show a higher adsorption ability within contact with pollutants. Additionally, the higher porosity contributes to oxygen between aggregate particles. Oxygen plays an essential role in plant growth and the existence of bacteria's in the rhizosphere. Thus, it has better conditions for pollutant removal and promotes aerobic respiration. Therefore CWs with the presents of the plants and correctly selected substrate show higher removal rates of pharmaceuticals, nutrient uptake than wetlands without vegetation (Lyu 2016).

5.2 Wastewater parameters

The parameters for the TOC and TC showed approximately the stable indicators for both types of substrates within all periods of study with insignificant differences in statistical data throughout the entire period (Figure 13; Figure 14). However, the parameters with sand filter have slightly risen in the mid-September period. Similar behavior in the same study period was observed when TC was measured. A significant difference in concentration has been noted between influent and effluent for both substrate samples. The concentration of TOC for sand fluctuated within 7.75-38.16 $\mu\text{g L}^{-1}$ and for perlite 5.97-22.45 $\mu\text{g L}^{-1}$.

The data for IC concentration for both sand and perlite have fixed undulated indicators throughout the entire term of the experiment, mainly with an increase in the first month and a decrease in the last month of sample collection (Figure 15). Moreover, a change was noted in descending order for both sand and perlite at the same period, mid-September. We didn't find a significant difference between influent and effluents data for both substrates. The outflow was highly saturated. The summary influent concentration was 820.64 $\mu\text{g L}^{-1}$, while the effluent data for sand and perlite was 631.07 $\mu\text{g L}^{-1}$ and 662.14 $\mu\text{g L}^{-1}$, respectively.

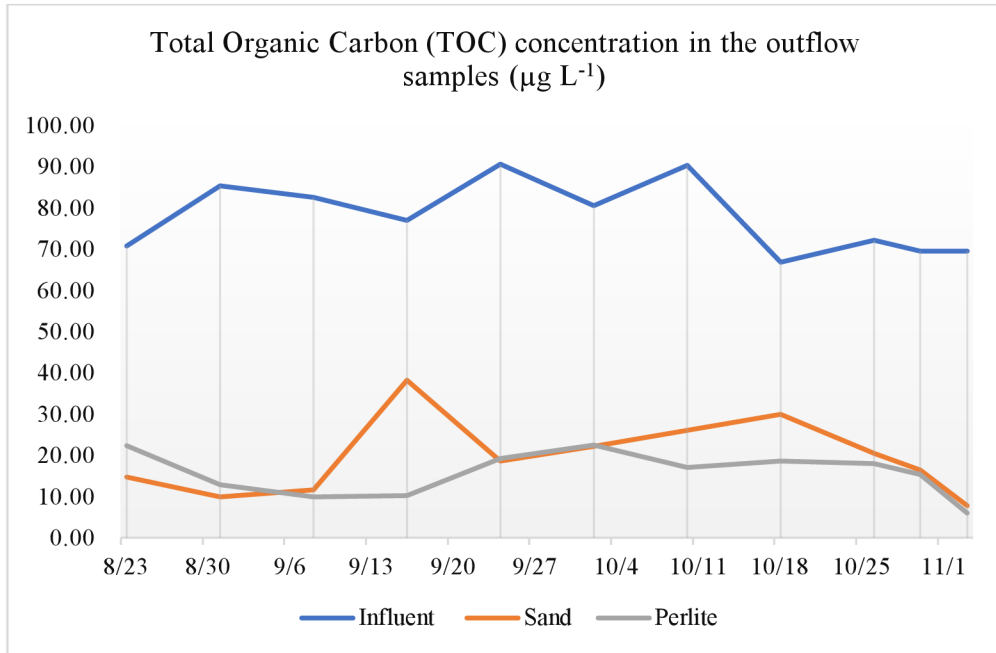


Figure 13. Total Organic Carbon (TOC) concentration in the outflow ($\mu\text{g L}^{-1}$)

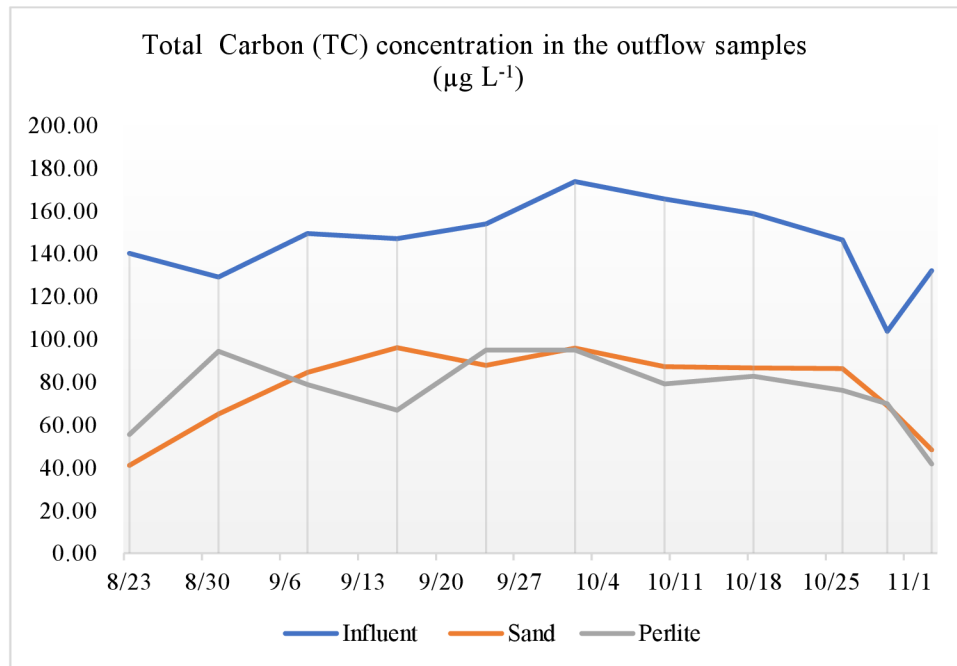


Figure 14. Total Carbon (TC) concentration in the outflow samples ($\mu\text{g L}^{-1}$)

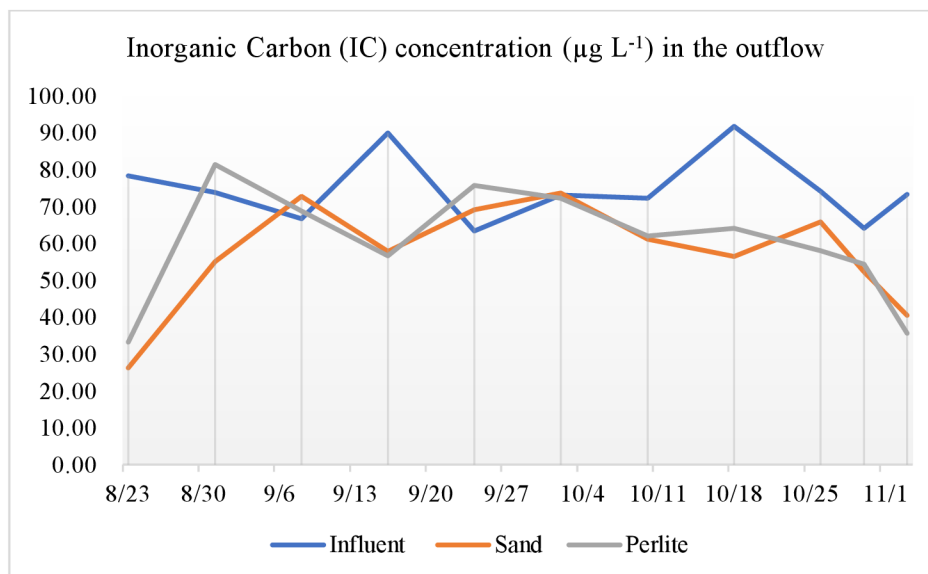


Figure 15. Inorganic Carbon (IC) concentration in the outflow ($\mu\text{g L}^{-1}$)

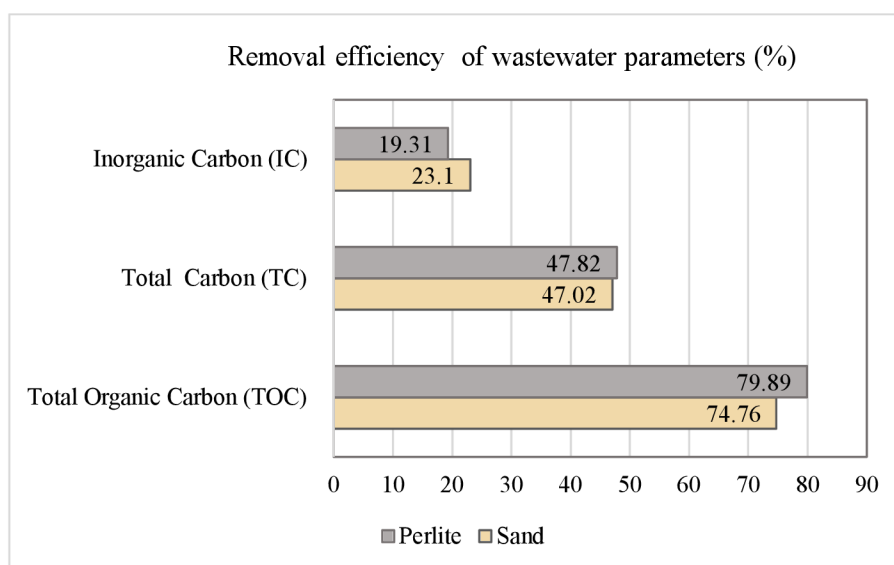


Figure 16. The removal efficiency of IC, TC, and TOC (%)

The removal efficiency for the TOC, TC, IC with the participation of the sandy sorbent was 74.76%, 47.02%, 23.1%, respectively. The TOC, TC, IC data was 79.89%, 47.82%, 19.31%, respectively, with the perlite as a substrate (Figure 16). The TOC for perlite illustrated the removal coefficient higher than sand and has higher indicators than TN and IC parameters. Ammonium (NH_4^+) concentration in the outflow for perlite during all periods was within the limit of 0.3-0.06 $\mu\text{g L}^{-1}$. Overall were stable throughout the whole period. The sand data was within the 4.17-4.12 $\mu\text{g L}^{-1}$ boundary,

which was higher than for the perlite. They did not have such stability and had abrupt changes in concentration (Figure 17). The removal efficiency reach averages 95.08% for perlite and 52.33% for sand. The conclusion follows from our data that perlite provides more success for ammonia removal compared to sand.

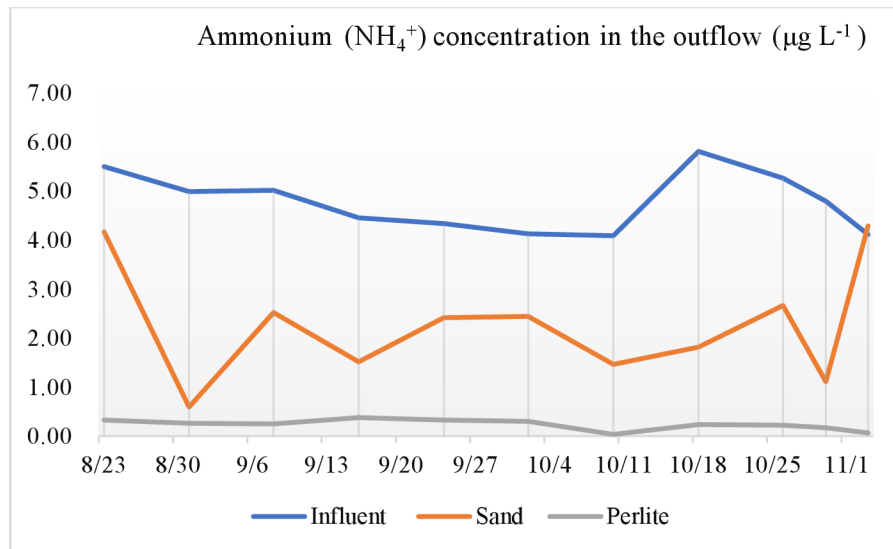


Figure 17. Ammonium (NH_4^+) concentration in the outflow ($\mu\text{g L}^{-1}$)

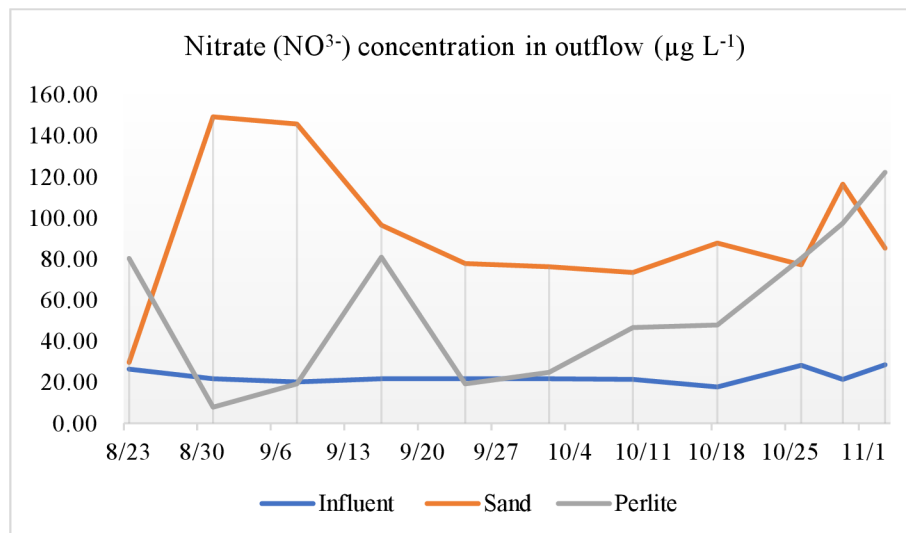


Figure 18. Nitrate (NO_3^-) concentration in the outflow ($\mu\text{g L}^{-1}$)

The concentration of Nitrate (NO_3^-) wasn't stable during all study periods (Figure 18). Samples with sand fluctuated within 29.63-85.23 $\mu\text{g L}^{-1}$. For the perlite

80.12-122.17 $\mu\text{g L}^{-1}$. Concentration for the sand was higher than for the perlite at the beginning of the study period. The amount leveled off towards the end of the experiment owing to a decrease in nitrate in sand samples. The overall picture for the component with perlite indicated a tendency to moderated increase over time.

The Nitrite (NO_2^-) revealed a sharp drop of concentration in the first month for both substrates, then, since the third month, was persistent in the stable lower volume throughout the remaining period (Figure 19).

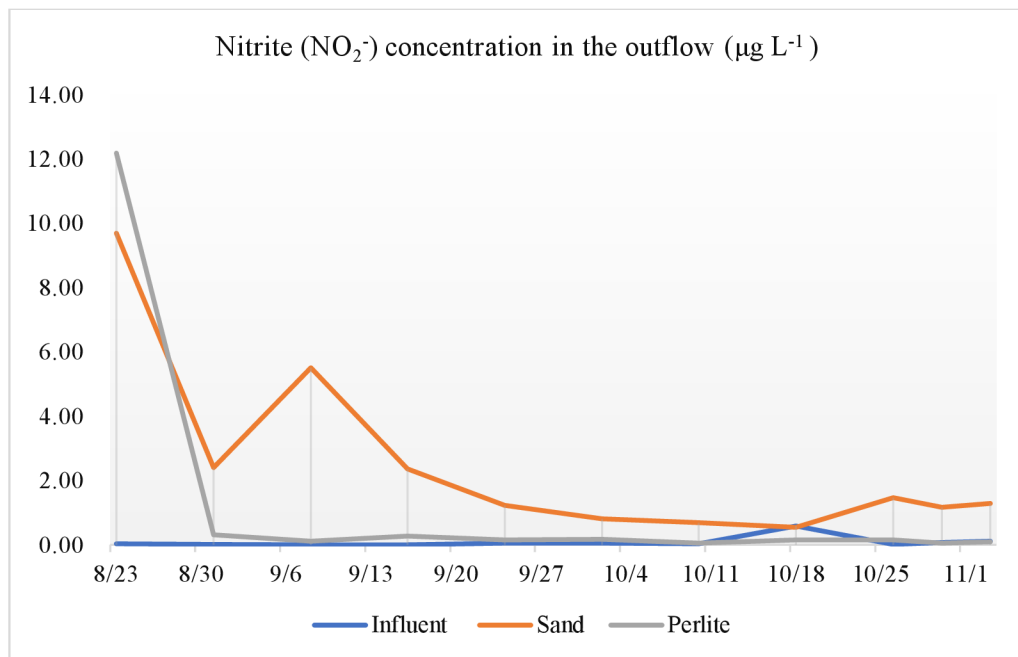


Figure 19. Nitrite (NO_2^-) concentration in the outflow ($\mu\text{g L}^{-1}$)

Phosphate (PO_4^{3-}) concentration in the effluent for the sand was higher than perlite within the whole study period, with slight fluctuation in indicators (Figure 20). This study revealed that both filter media sand and perlite efficiently removed phosphorus from water, mainly at the beginning of the experiment.

The removal efficiency of the Ammonium (NH_4^+) and Phosphate (PO_4^{3-}) with the participation of the sandy sorbent was 52.33% and 53.75%, respectively. The data with the perlite as a substrate was 95.08% and 79.07%, respectively (Figure 21).

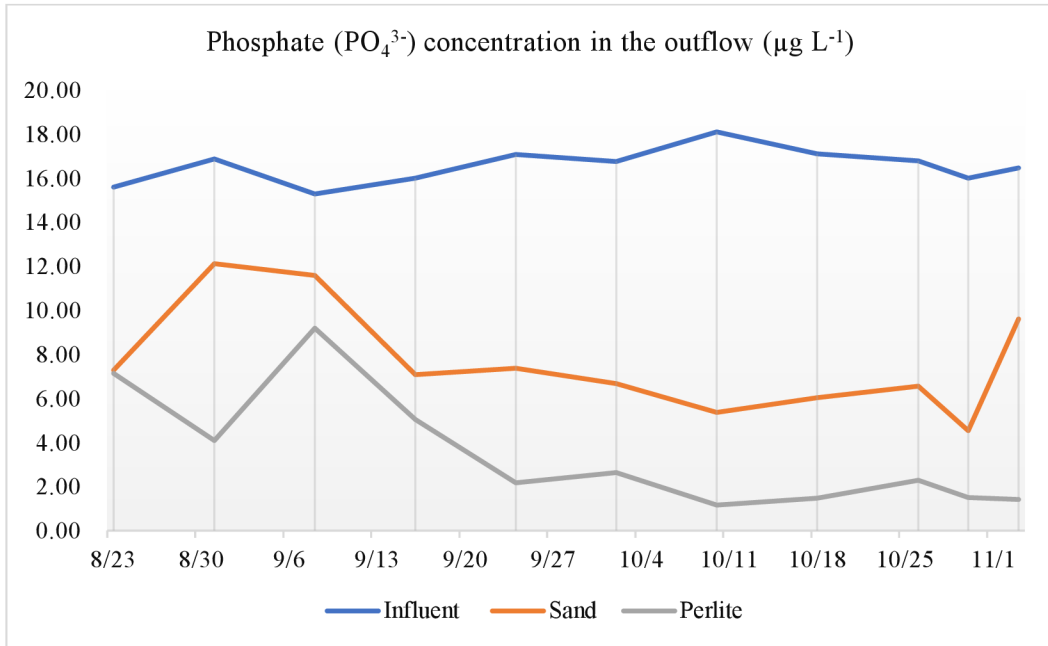


Figure 20. Phosphate (PO_4^{3-}) concentration in the outflow ($\mu\text{g L}^{-1}$)

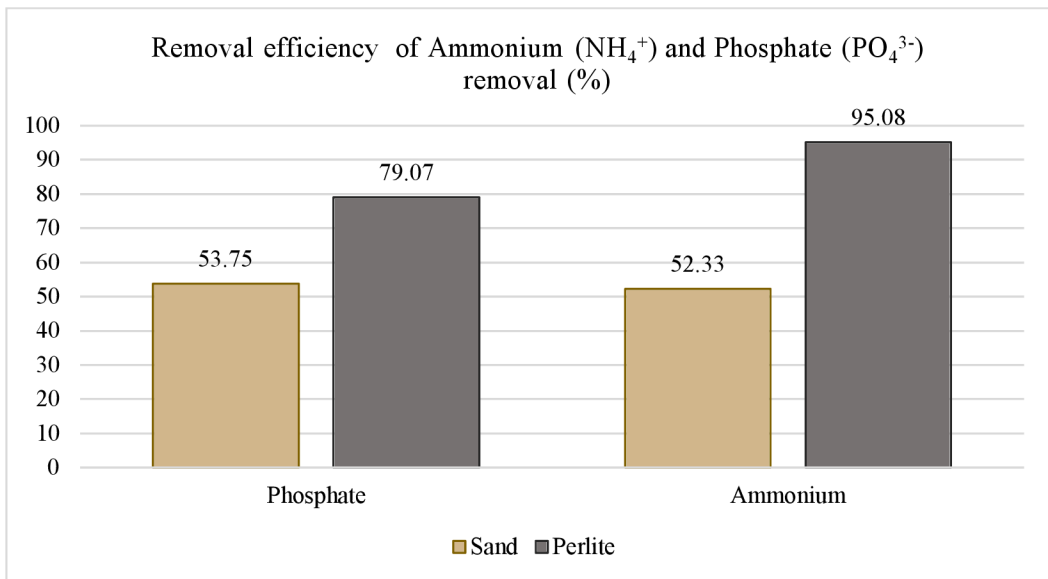


Figure 21. The removal efficiency of Ammonium (NH_4^+) and Phosphate (PO_4^{3-}) (%)

5.3 Ibuprofen and Diclofenac in the effluent

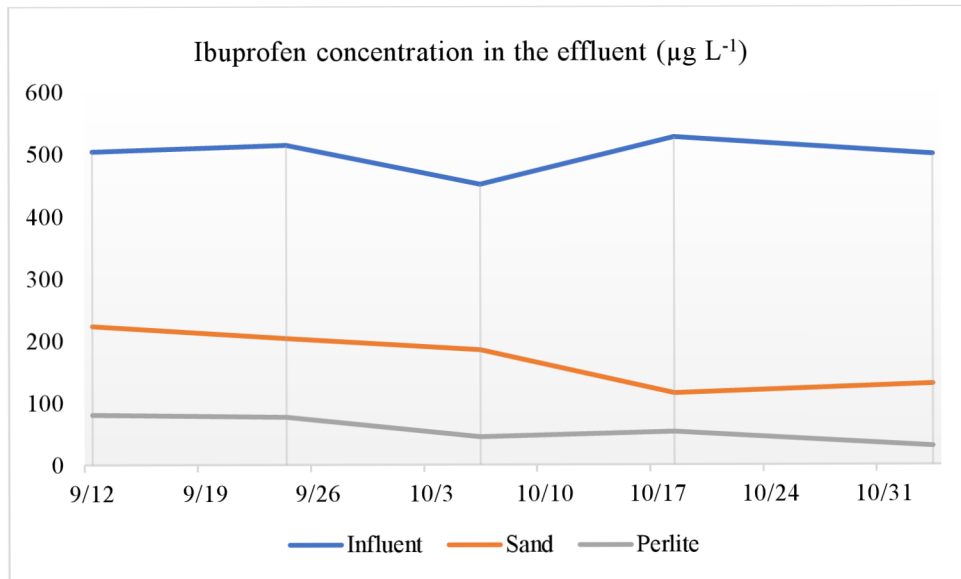


Figure 22. Ibuprofen in the outflow ($\mu\text{g L}^{-1}$)

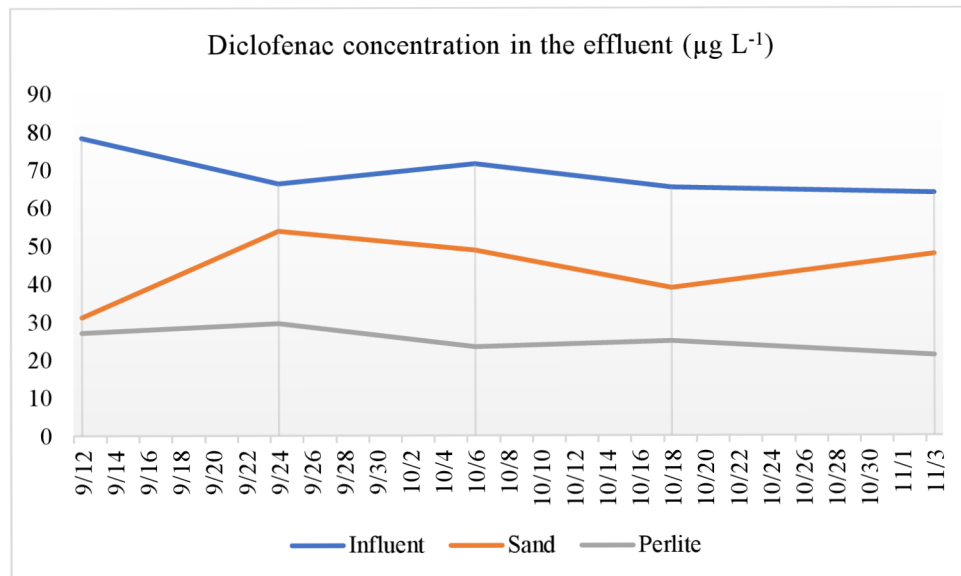


Figure 23. Diclofenac concentration in the effluent ($\mu\text{g L}^{-1}$)

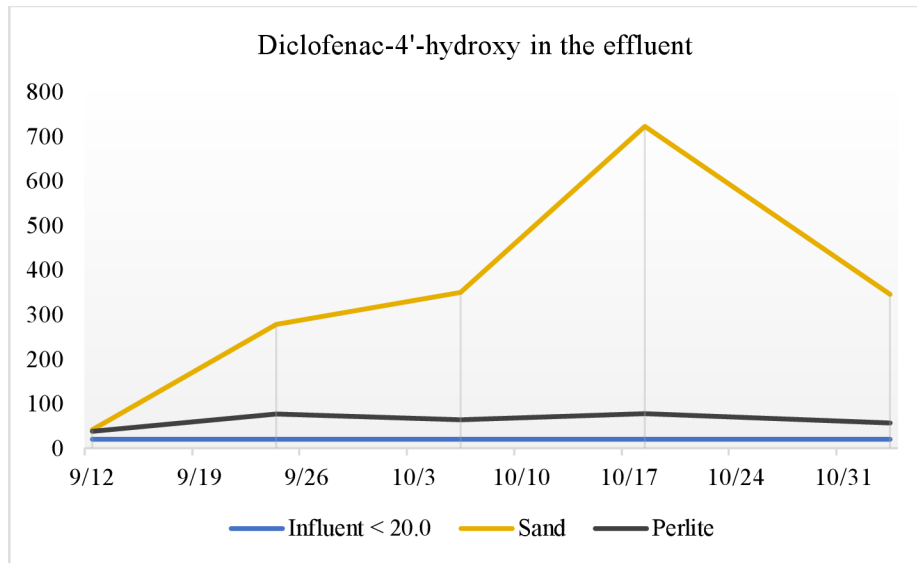


Figure 24. DCF metabolite: Diclofenac-4'-hydroxy in the effluent ($\mu\text{g L}^{-1}$)

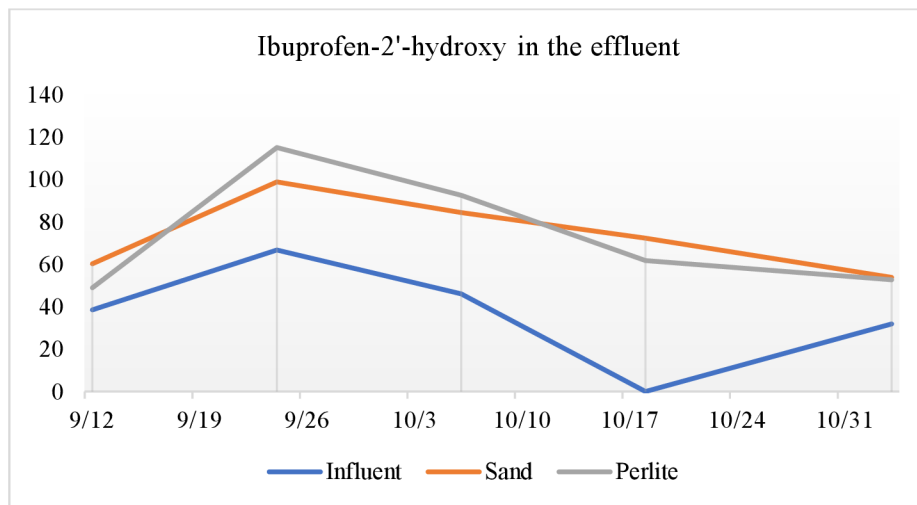


Figure 25. IBU metabolite: Ibuprofen-2'-hydroxy in the effluent ($\mu\text{g L}^{-1}$)

The amount of IBU and DCF in the effluent showed higher indicators for the sand versus perlite. The data showed a relatively similar amount in outflow samples throughout the experimental period (Figure 22; Figure 23). Also, data for the Diclofenac-4'-hydroxy in the effluent showed higher numbers for the sand (Figure 24). The data for the Ibuprofen-2'-hydroxy indicates a relatively similar level, with an increase in volumes in September and a gradual slight decline in the subsequent months (Figure 25).

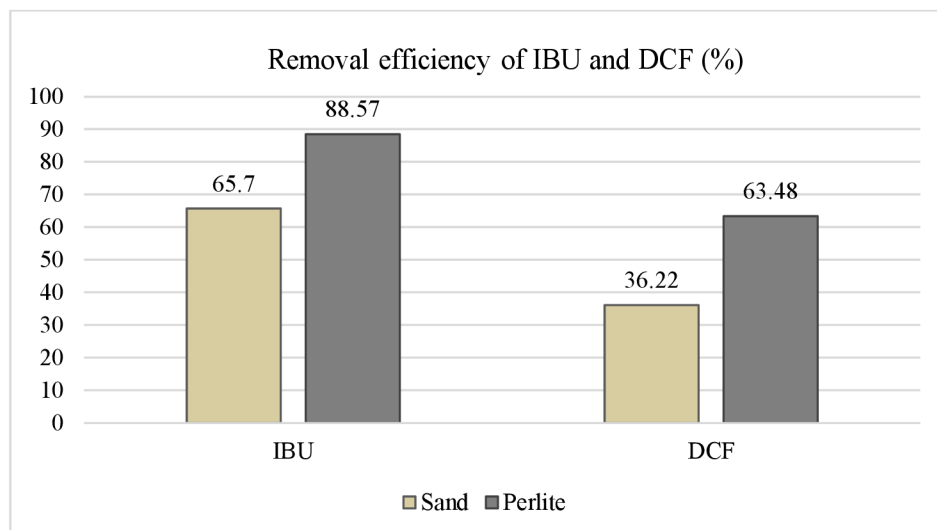


Figure 26. The removal efficiency of IBU and DCF (%)

The removal efficiency of the IBU for the sandy and perlite was 65.7%, 88.57%, respectively. The removal efficiency of DCF for sand and perlite as a substrate was 36.22% and 63.48%. The difference in removal efficiency showed significant gaps. The sand was less effective than perlite for IBU by 22.87%, while for DCF its efficiency comparison to perlite, was lower by 27.26%. Hence, as illustrated by Figure 26, perlite demonstrated an appreciable contribution to IBU and DCF removal.

5.4 Ibuprofen and Diclofenac in the plant roots and rhizosphere soil and plant roots

Table 6. Ibuprofen and Diclofenac content and their metabolites in the rhizosphere soil ($\mu\text{g}/\text{kg}$)

| <i>Compounds</i> | <i>Sand</i> | <i>Perlite</i> |
|--|-----------------|--------------------|
| <i>Ibuprofen (IBU)</i> | <i><10,0</i> | <i>11.3</i> |
| <i>2-hydroxy ibuprofen (2-OH-IBU)</i> | <i><20,0</i> | <i><20,0</i> |
| <i>Carboxy ibuprofen (CA IBU)</i> | <i><5,0</i> | <i><5,0</i> |
| <i>Diclofenac (DCF)</i> | <i><10,0</i> | <i><10,0</i> |
| <i>4'-hydroxy diclofenac (4'-OH DCF)</i> | <i><20,0</i> | <i><20,0</i> |

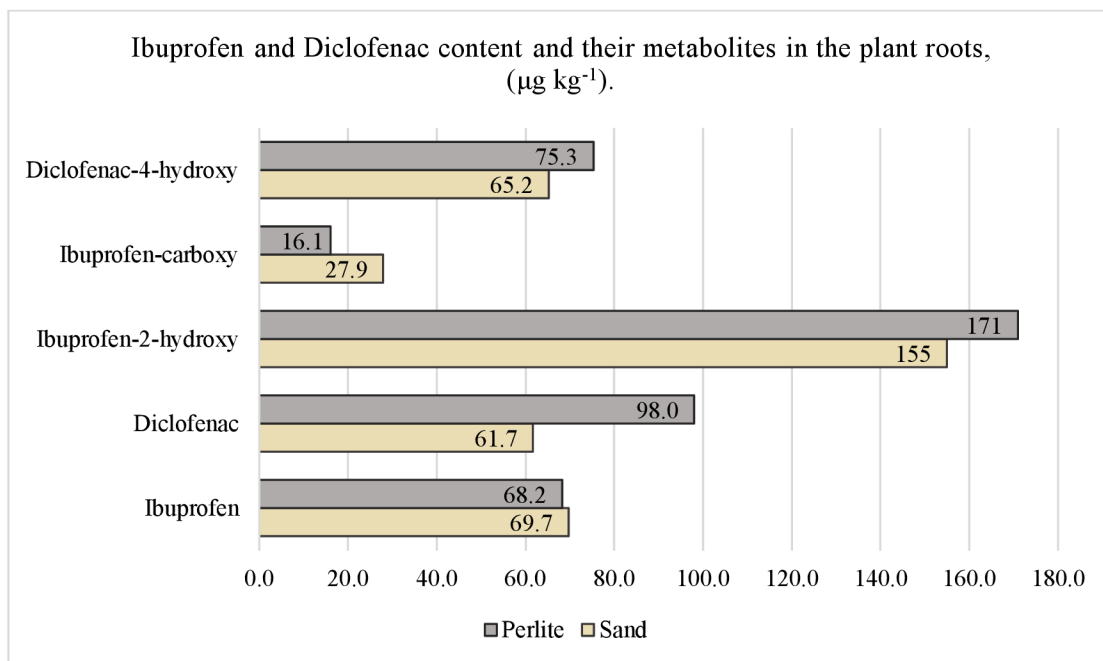


Figure 27. The content of Ibuprofen and Diclofenac and their metabolites in the plant roots ($\mu\text{g kg}^{-1}$)

The investigation of the content of metabolites in the rhizosphere indicates the following data. The pharmaceutical's content and metabolites in the rhizosphere for sand soil showed results for all compounds within non-exceeded limits. The Ibuprofen-2-hydroxy indicates $<20.0 \mu\text{g kg}^{-1}$ for the rhizosphere using both types of substrates. Ibuprofen-carboxy was $<5.0 \mu\text{g kg}^{-1}$ and Diclofenac-4-hydroxy $<20.0 \mu\text{g kg}^{-1}$. Only the content of IBU in rhizosphere soil with perlite reported data is $11.3 \mu\text{g kg}^{-1}$, which is higher than detection limited (Table 6). Overall, the contents in the rhizosphere had no differences between sand and perlite systems.

The analysis estimated for the plant roots for the pharmaceuticals and their metabolites demonstrated results: the sand data showed a content of IBU $69.7 \mu\text{g kg}^{-1}$, which is higher than for perlite $68.2 \mu\text{g kg}^{-1}$. On the other hand, the sand with the content of DCF was $61.7 \mu\text{g kg}^{-1}$, which was much lower than for perlite $98.0 \mu\text{g kg}^{-1}$. Also, the content for the metabolites such as Ibuprofen-2-hydroxy and Diclofenac-4-hydroxy for perlite was higher than for sand. But, Ibuprofen-carboxy data for perlite was lower. From the figure, we can conclude that IBU content in the plant was mostly the same for both types of substrates. At the same time, the DCF amount was considerably higher for perlite than for the sand. So, the average data for the plant root using perlite as a sorbent was higher than for the sand (Figure 27).

6. Discussion

The results of experiments have shown that one of the fundamental indicators of a plant's life is receiving all nutrients and water using roots. It involves qualitative change where an adsorbed material can be transformed and becomes a tissue resulting in the production of a new mass of the aboveground part of the plants. Nutrient uptake by roots also leads to an increase in the surface area. The wetland plants have shown successful results in domestic wastewater treatment. As reported, plants may act as an “engine” for nutrient uptake, raising the diversity in the rhizosphere and enhancing biological and chemical reactions in terms of supporting water purification (Vymazal 2007). The soil structure plays the primary role as supporting material for plant growth and microbial community interaction. In addition, microbes are essential for various ecological and physiological functions, including regulating mineral nutrient availability, decomposition, and producing biologically active substances.

The five months of study have demonstrated by our results that the type of substrates may play a significant role and have a considerable influence on the biomass of plants. The given outcome has an agreement with Stottmeister et al. (2003) that soil has a crucial aspect of the interaction taking place in the rhizosphere, such as interconnection and synergistic effect between rhizomes and the soil matrix. Additionally, to ensure the stable performance of the VSSF, there is the requirement for suitable material as a substrate to prevent clogging of the system (Yang et al., 2018). Therefore, lightweight substrate perlite illustrates the difference in the outcome by its physical properties for wastewater treatment in CWs. Due to its porous structure and higher oxygen diffusion, perlite keeps the soil loose, light, and aerated. Furthermore, its physical parameters and composition influence all systems, such as internal spaces, particle sizes, and irregularity. These factors contribute to circulating air between the roots for healthy plant growth.

Additionally, the aeration between particles provides suitable environmental conditions for the microbial. Therefore, it promotes root growth and increases the photosynthetic rate and chlorophyll content in the plant, improving their life cycle. In particular, the perlite has proved a dominant effect on the root growth compared to the sand (Figure 11). Furthermore, since perlite is a mesoporous material, it significantly affects the average development of plant biomass (Petrella et al., 2018) (Figure 12).

Despite perlite being more porous than sand, it has less ability to retain water. But, at the same time, it creates a good condition for oxygen diffusion. Thus, in total, perlite as sorbent can contribute higher compared to the sand removal process of contaminants. In addition, the previous authors reported that soil with limited access to aeration inside the soil's porous volume increases CO₂ concentration with a temporary rise of pH in the rhizosphere. Perlite demonstrates the ideal balance between air and water (Markoska, Spalevic, and Gulaboski 2018). Nevertheless, it is worth considering the value of total porosity in order to provide the plant with sufficient quantities of water and air.

The sum parameter of TOC in the outflow for EPs demonstrated different indicators for both substrate types. The removal efficiency for sand as the substrate has shown 74.76%. But the perlite parameters for TOC had a higher efficiency, with 79.89% (Figure 16). It can be explained that TOC levels in water increase upon exposure to air. TOC is an indicator of water quality measured by analyzing the organic contamination in a water sample. It has good agreement with the study of Sleytr et al. (2007). It was noted that due to intermittently loading and type of CWs, the high removal efficiency of TOC can be explained by dependency on oxygen as an essential parameter for aerobic biological processes. The aeration can also be suitable and promote the expansion of the microbial biomass, which plays a crucial role in the bioremediation process and transforms nutrients. In our experiment, perlite as a more aerated material has shown promising results for the TOC removal.

The results demonstrated the substrate's relatively stable efficiency for TC (Figure 14). On the other side, the concentration of IC constantly changed throughout the whole experimental period, and no significant difference was revealed in the inflow and outflow (Figure 15). It may be assumed that IC is impacted by soil's biotic respiration processes, temperature, nutrient retention, microbial interaction, and alteration of the soil pH (Dodds 2002). Besides, the presence of plants and their efficiency on the removal process, as plant uptake of organic carbon and nutrient showed no significant difference between planted and no planted CWs (Hidayah, Chou, and Yeh 2018). Thus, the differences in indicators for IC and dependence water parameters on various factors should be further explored.

Ammonium concentration with the participation of different substrates has displayed a substantial difference in the data. Thus, perlite indicated the active role of sorption for the pollutant within the study. The results of our data have shown that perlite influences more successfully for ammonia removal compared to sand (Figure 17; Figure 21). It can be explained that perlite, due to its porosity and ability to retain a liquid, provides a conducive condition for oxygen diffusion and, consequently, for an essential element for plant growth. On the other side, the high concentration of NH_4^+ along with air stripping, ion exchange, and biological nitrification-denitrification can cause a sharp decrease in dissolved oxygen (Zhu et al., 2011; Seruga et al., 2019). Within the process of uptaking ammonia and nitrate by macrophytes, the inorganic N forms convert into organic compounds and serve as a building material for cells and tissues (C. G. Lee, Fletcher, and Sun 2009). The adsorbed ammonia can be released quickly depending on the changes in water chemistry conditions. Decreasing ammonia concentration in the water column reduces the result of nitrification. On the contrary, increasing the ammonia concentration in the water column will also increase the adsorbed ammonia. Thus, the CWs process should consider the interaction between nutrient levels, water quality conditions, dissolved oxygen concentrations, plants, and substrate. However, including the obtained results, it is worth considering that ammonium can be inhibited by pH change, toxic shock, low-dissolved oxygen, and temperature decrease.

The study results indicate the presence of the concentration of Nitrate (NO_3^-) in sandy substrates in higher concentrations than perlite. The content of perlite demonstrates a lower amount. Only at the end of the study was there a rising (Figure 18). It can be assumed that all these changes occurred due to external factors. Nitrification is a chemoautotrophic process. Here is the biological oxidation of ammonium can be converted to nitrate enormously depending on indicators of temperature and pH. Due to neutral pH parameters, perlite is defined as an effective sorbent for the removal of nitrate from aqueous solutions. In turn, pH is very dependent on temperature (Baei, Mazyar Sharifzadeh 2009). With it declining, the pH is rising. Thus, as we can see from the perlite result, the efficiency of adsorption decreases accordingly with decreasing temperature.

According to the results, the Nitrite (NO_2^-) in the effluent has shown in shallow concentration. Data were recorded in the particular beginning from the second month

of the experiment (Figure 19). The dependency of nitrite pollution can probably explain it from the temperature. Nitrite is very dangerous for human health, such as activating the efflux of potassium from muscle and erythrocytes and compromising blood transport (Jensen 2003). Thus, one of the vital tasks, it nitrite removal. Nitrite adsorption with the sand and perlite can be used in the future wastewater treatment plant.

Plants in CWs can take up nitrogen and phosphate as nutrition. Meanwhile, plants provide a suitable condition for nitrification and denitrification. Additionally, vegetation contributes to an anaerobic and aerobic living environment in the rhizosphere, which ensures requirements for the rate of nitrogen removal. Moreover, planted CWs tends to remove nitrate more effectively than non-vegetated wetland system. It was noted that nitrate could be successfully removed without the participation of carbon in case the macrophytes are present in wetlands (LU et al., 2009). Therefore, rising plant density may affect the decline in nitrogen concentration within the system. Furthermore, vegetation provides a surface area for microbial growth, thus increasing nitrification and denitrification (Raharjo et al., 2015).

Phosphorus is a significant nutrient contaminant and a critical element that causes eutrophication of the surface water. Our results are in agreement with previous research regarding filters. Brix et al. (2001) also showed relatively low phosphorus sorption capacities of different soil types. Moreover, it has been noted that wetlands have a finite ability to absorb phosphorus. After a period of usage, sorbent stops adsorbing pollutants once saturated, which causes an efficiency removal decrease (Ballantine and Tanner 2010; (Loganathan et al., 2014).

Furthermore, phosphorus (P) may become a P source when physicochemical conditions change. Therefore, it was suggested for the sand that adding other chemical substances could significantly enhance the sorption capacity. Additionally, the better elimination of P requires a large wetland and long residence times.

The laboratory-scale with perlite as the filter media was, on average by 25% more efficient in total (PO_4^{3-}) removal than the sand media (Figure 20). Thus, the investigation results suggest using perlite as a better option since substrates sand are less effective applicants for long-term treatment, as shown in the study (Figure 21).

Our results for IBU removal efficiency with perlite substrates have shown 88,57% (Figure 26) and agree with previous research by de Oliveira et al. (2019), indicating the EPs removal efficiency in the VFCW 89% for IBU. The Oliveira et al. (2019) study emphasized that the VFCW system with filter media achieved better removal results for IBU than the FFM-CW (free-water surface constructed wetlands). In our research, IBU and DCF in the effluent demonstrated the same stable concentration but dominant efficiency results for the perlite from the beginning until the end of the analysis (Figure 22; Figure 23). Pharmaceuticals IBU, DCF, and their metabolites showed higher content using perlite as a sorbent. The perlite promotes the suitable condition for the balance of water-oxygen due to its porous structure. Thus, perlite by increasing oxygen content and aerobic conditions can contribute to the more efficient results regarding IBU (Yang Zhang et al., 2017).

Additionally, pharmaceuticals' hydrophobic characteristics, aeration, and solubility should be considered. IBU has medium water solubility ($21 \mu\text{g L}^{-1}$), hydrophobic characteristics, and high mobility in the water. In turn, DCF has high water solubility ($5000 \mu\text{g L}^{-1}$) (Bhadra et al., 2017). Based on the analysis of the plant growing, it can be concluded that plant uptake is more efficient with the participation of perlite as it provides oxygen for the plant's growth (Figure 27). Coupled with the parameters mentioned above, the removal, in the study by Yang Zhang et al. (2017), was proven that efficiency depended on the plant species used in the CWs and season (for IBU) and initial additives concentration. IBU elimination is highly dependent on plants' oxygenation of the water (de Oliveira et al., 2019). Therefore, it may be noted that *G. maxima* can ensure a favorable treatment condition and contribute to an IBU elimination. Also, it was suggested by the author Yang Zhang et al. (2017) that the high removal efficiency of IBU, together with the low rates of phytoaccumulation and sorption to the substrate, was the main purification pathway for IBU in the saturated CW mesocosms. In addition, the removal of IBU gets better results at high temperature and aerobic conditions.

The metabolize of Diclofenac-4'-hydroxy in effluent reported a higher concentration for sand increased toward the end of the study (Figure 24). In addition, the excess content of 4'-OH DCF in the sand samples increased with time, probably due to the low aeration or solubility of DCF in the water. IBU and DCF content and their metabolites in rhizosphere soil showed indicators below the detected limit in

almost all cases. The only data for the IBU using perlite has an exceeded rate (Table 6). Overall, the sorption degree of EPs in a substrate was found to be dependent on the chemical structures and types of different compounds. Besides, planted CWs have proven their contribution and show higher removal efficiency of pharmaceuticals. However, the adsorption process of medications is still very little understood and requires study due to the urgent need to preserve environmental conditions and water quality. Nevertheless, based on the results, the sorption process in CWs can be considered to be an effective water treatment option, particularly the use of perlite-based sorbent is very promising.

7. Conclusions

Many emerging pollutants are released continuously into the environment from various anthropogenic sources, thereby endangering the environment in chronic toxicity, endocrine disruption in humans and the aquatic system. The need for environmentally friendly treatment methods was embodied in CWs, which act as a biofilter allowing to remove a range of contaminants. The thesis devised above aimed to identify and compare the adsorption efficiency of emerging pollutants, including pharmaceuticals, for two separate substrates, sand and perlite. The analysis, which used small-scale experimental VFCWs, illustrates different results for each compound. The experiment has shown that perlite as adsorptive substrates plays a critical function in CWs. Due to its porous structure, perlite contributed to the faster growth of wetland plants. It promoted the development of all plant tissue, thereby enhancing the surface area for absorption metabolites, which was beneficial by the plant nutrient uptake process. This study indicated that the inclusion of substrates perlite and sand had increased the removal rates of organic compounds, with perlite being the most efficient in TC and TOC removal. On the other hand, removing IC using the perlite as an adsorbent was less efficient than sand. Therefore, further study should be conducted because IC is impacted by climate conditions, including temperature and soil pH alteration. Perlite has shown positive effects in NH_4^+ and PO_4^{3-} treatment and demonstrated over 79 and 95% removal efficiencies, respectively. The removal efficiency of IBU and DCF was higher in columns with perlite and indicated 88.57% and 63.48%, respectively, which can be explained due to the aerobic condition of

substrate, structure, and hydrophobic properties of sorbent. Exceed of the content of IBU in the rhizosphere can be explained by increased solubility of IBU and at the same time by releasing of root exudates that contribute to pharmaceutical degradation.

However, the overall picture is not yet clear enough since there are still multiply gaps in the study of the toxicology of pharmaceuticals, their transformation products, and their interaction with other inorganic contaminants in the environment. Furthermore, the shortcoming of our experiment was that it was carried out in a given climatic region limited by the climatic conditions of the current season, in particular temperature, lighting, and humidity. Moreover, the measurement error should be taken into account since perlite is only one of the chain elements in the removal process. Additionally, microbial interaction was not evaluated, and the synergistic/antagonistic effect of all participants involved in a given remediation process was also not considered.

Thus, the study conducted above assists in getting an expanded practical knowledge concerning the aspect involved in the adsorption mechanism. It can be summarized that substrate plays a crucial role in the removal process. Our study has demonstrated that perlite coped better with issues and obstacles than the sandy filter. Therefore, it has the potential to expand its usage in the adsorption process. Unfortunately, perlite is a non-renewable resource since it naturally occurs. Nevertheless, due to low cost, reuse ability, perlite is the most promising filter material in removing given emerging pollutants, including pharmaceuticals.

8. Bibliography

- 2000/60/EC, DIRECTIVE. 2000. "Pravention Lokaler Schleimhautlasionen Wahren Oropharynx-Bestrahlung Durch Einen Plombenschutz." *Strahlentherapie Und Onkologie* 168 (1): 35–38.
- Ahmed, Sarfraz, Muhammad Ibrahim, Fiaz Ahmad, Hafsa Anwar Rana, Tazeen Rao, Wajiha Anwar, Muhammad Younus, et al. 2019. *Microbial Risk Assessment and Antimicrobial Resistance. Antibiotics and Antimicrobial Resistance Genes in the Environment: Volume 1 in the Advances in Environmental Pollution Research Series*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-818882-8.00020-6>.
- Almuktar, Suhad A.A.A.N., Suhail N. Abed, and Miklas Scholz. 2018. "Wetlands for Wastewater Treatment and Subsequent Recycling of Treated Effluent: A

- Review.” *Environmental Science and Pollution Research* 25 (24): 23595–623. <https://doi.org/10.1007/s11356-018-2629-3>.
- Amos Sibeko, Pheko, Devrani Naicker, Phumlane Selby Mdluli, and Lawrence Mzukisi Madikizela. 2019. “Naproxen, Ibuprofen, and Diclofenac Residues in River Water, Sediments and Eichhornia Crassipes of Mbokodweni River in South Africa: An Initial Screening.” *Environmental Forensics* 20 (2): 129–38. <https://doi.org/10.1080/15275922.2019.1597780>.
- Aneyo, Idowu A., Funmilayo V. Doherty, Olumide A. Adebisin, and Mariam O. Hammed. 2016. “Biodegradation of Pollutants in Waste Water from Pharmaceutical, Textile and Local Dye Effluent in Lagos, Nigeria.” *Journal of Health and Pollution* 6 (12): 34–42. <https://doi.org/10.5696/2156-9614-6.12.34>.
- Baei, Mazyar Sharifzadeh, Hossein Esfandia. 2009. “Removal of Nitrate from Aqueous Solutions in Batch Systems using Activated Perlite: An Application of Response Surface Methodology.” *Technology*, no. 17: 743–53. <https://doi.org/10.1002/apj>.
- Bali, Mahmoud, and Moncef Gueddari. 2019. “Removal of Phosphorus from Secondary Effluents Using Infiltration–Percolation Process.” *Applied Water Science* 9 (3): 1–8. <https://doi.org/10.1007/s13201-019-0945-5>.
- Ballantine, Deborah J., and Chris C. Tanner. 2010. “Substrate and Filter Materials to Enhance Phosphorus Removal in Constructed Wetlands Treating Diffuse Farm Runoff: A Review.” *New Zealand Journal of Agricultural Research* 53 (1): 71–95. <https://doi.org/10.1080/00288231003685843>.
- Bartz, Wilfried J. 1998. “Chapter 7 Environmental Issues.” *Tribology and Interface Engineering Series* 45 (i): 267–300. [https://doi.org/10.1016/S0167-8922\(03\)80022-6](https://doi.org/10.1016/S0167-8922(03)80022-6).
- Bastani, D., A. A. Safekordi, A. Alihosseini, and V. Taghikhani. 2006. “Study of Oil Sorption by Expanded Perlite at 298.15 K.” *Separation and Purification Technology* 52 (2): 295–300. <https://doi.org/10.1016/j.seppur.2006.05.004>.
- Bell, Katherine Y, Martha J M Wells, Kathy A Traexler, Marie-laure Pellegrin, Audra Morse, and Jeff Bandy. 2011. “Emerging Pollutants” 83 (10): 1906–84.
- Bhadra, Biswa Nath, Imteaz Ahmed, Sunghwan Kim, and Sung Hwa Jung. 2017. “Adsorptive Removal of Ibuprofen and Diclofenac from Water Using Metal-Organic Framework-Derived Porous Carbon.” *Chemical Engineering Journal* 314: 50–58. <https://doi.org/10.1016/j.cej.2016.12.127>.
- Bhosle, Aureen Godinho and Saroj. 2013. “Rhizosphere Bacteria from Coastal Sand Dunes and Their Applications in Agriculture.” *Bacteria in Agrobiolology: Crop Productivity*, 1–507. <https://doi.org/10.1007/978-3-642-37241-4>.
- Boxall, Alistair B. 2012. “New and Emerging Water Pollutants,” 49.
- Brisson, J., and F. Chazarenc. 2009. “Maximizing Pollutant Removal in Constructed Wetlands: Should We Pay More Attention to Macrophyte Species Selection?” *Science of the Total Environment* 407 (13): 3923–30. <https://doi.org/10.1016/j.scitotenv.2008.05.047>.
- Brix, H., C. A. Arias, and M. Del Bubba. 2001. “Media Selection for Sustainable

- Phosphorus Removal in Subsurface Flow Constructed Wetlands.” *Water Science and Technology* 44 (11–12): 47–54.
<https://doi.org/10.2166/wst.2001.0808>.
- Brix, Hans. 1997. “Do Macrophytes Play a Role in Constructed Treatment Wetlands?” *Water Science and Technology* 35 (5): 11–17.
[https://doi.org/10.1016/S0273-1223\(97\)00047-4](https://doi.org/10.1016/S0273-1223(97)00047-4).
- . 2003. “Plants Used in Constructed Wetlands and Their Functions.” *I St. International Seminar on the Use of Aquatic Macrophytes for Wastewater Treatment in Constructed Wetlands*, no. December: 30.
- Brooks, Bryan W., Christy M. Foran, Sean M. Richards, James Weston, Philip K. Turner, Jacob K. Stanley, Keith R. Solomon, Marc Slattery, and Thomas W. La Point. 2003. “Aquatic Ecotoxicology of Fluoxetine.” *Toxicology Letters* 142 (3): 169–83. [https://doi.org/10.1016/S0378-4274\(03\)00066-3](https://doi.org/10.1016/S0378-4274(03)00066-3).
- Bruch, I., U. Alewell, A. Hahn, R. Hasselbach, and C. Alewell. 2014. “Influence of Soil Physical Parameters on Removal Efficiency and Hydraulic Conductivity of Vertical Flow Constructed Wetlands.” *Ecological Engineering* 68: 124–32.
<https://doi.org/10.1016/j.ecoleng.2014.03.069>.
- Calheiros, Cristina S.C., Anouk F. Duque, Alexandra Moura, Isabel S. Henriques, António Correia, António O.S.S. Rangel, and Paula M.L. Castro. 2009. “Changes in the Bacterial Community Structure in Two-Stage Constructed Wetlands with Different Plants for Industrial Wastewater Treatment.” *Bioresource Technology* 100 (13): 3228–35.
<https://doi.org/10.1016/j.biortech.2009.02.033>.
- Carberry, Judith Bower, and John Wik. 2001. “Comparison of Ex Situ and in Situ Bioremediation of Unsaturated Soils Contaminated by Petroleum.” *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering* 36 (8): 1491–1503. <https://doi.org/10.1081/ESE-100105726>.
- Cleuvers, Michael. 2004. “Mixture Toxicity of the Anti-Inflammatory Drugs Diclofenac, Ibuprofen, Naproxen, and Acetylsalicylic Acid.” *Ecotoxicology and Environmental Safety* 59 (3): 309–15. [https://doi.org/10.1016/S0147-6513\(03\)00141-6](https://doi.org/10.1016/S0147-6513(03)00141-6).
- Corada-Fernández, Carmen, Lucila Candela, Nivis Torres-Fuentes, Marina G. Pintado-Herrera, Maria Paniw, and Eduardo González-Mazo. 2017. “Effects of Extreme Rainfall Events on the Distribution of Selected Emerging Contaminants in Surface and Groundwater: The Guadalete River Basin (SW, Spain).” *Science of the Total Environment* 605–606: 770–83.
<https://doi.org/10.1016/j.scitotenv.2017.06.049>.
- D’Ascenzo, G., Antonio Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, and R. Samperi. 2003. “Fate of Natural Estrogen Conjugates in Municipal Sewage Transport and Treatment Facilities.” *Science of the Total Environment* 302 (1–3): 199–209. [https://doi.org/10.1016/S0048-9697\(02\)00342-X](https://doi.org/10.1016/S0048-9697(02)00342-X).
- Debska, Anna, Krzysztof Józwiakowski, Magdalena Gizińska-Górna, Aneta Pytka,

- Michał Marzec, Bożena Sosnowska, and Agata Pieńko. 2015. "The Efficiency of Pollution Removal from Domestic Wastewater in Constructed Wetland Systems with Vertical Flow with Common Reed and *Glyceria Maxima*." *Journal of Ecological Engineering* 16 (5): 110–18. <https://doi.org/10.12911/22998993/60464>.
- Directive. 2008. "Water Environmental Quality Standards." *CONCAWE Review* 16 (1): 11–14.
- Dodds, Walter K. 2002. "Freshwater Ecology, 2002" 2002. <https://doi.org/10.1016/B978-0-12-219135-0.50013-1>.
- Dordio, Ana V., José Teimão, Idália Ramalho, A. J. Palace Carvalho, and A. J. Estêvão Candeias. 2007. "Selection of a Support Matrix for the Removal of Some Phenoxyacetic Compounds in Constructed Wetlands Systems." *Science of the Total Environment* 380 (1–3): 237–46. <https://doi.org/10.1016/j.scitotenv.2007.02.015>.
- Dulio, Valeria, Bert van Bavel, Eva Brorström-Lundén, Joop Harmsen, Juliane Hollender, Martin Schlabach, Jaroslav Slobodnik, Kevin Thomas, and Jan Koschorreck. 2018. "Emerging Pollutants in the EU: 10 Years of NORMAN in Support of Environmental Policies and Regulations." *Environmental Sciences Europe* 30 (1). <https://doi.org/10.1186/s12302-018-0135-3>.
- Eggen, Trine, and Christian Vogelsang. 2015. "Occurrence and Fate of Pharmaceuticals and Personal Care Products in Wastewater." *Comprehensive Analytical Chemistry* 67: 245–94. <https://doi.org/10.1016/B978-0-444-63299-9.00007-7>.
- Eskander, Samir, and Hosam El-din Mostafa Saleh. 2017. "Biodegradation : Process Mechanism." *Biodegradation and Bioremediation* 8 (January): 1–31.
- Estévez, Esmeralda, María del Carmen Cabrera, Antonio Molina-Díaz, José Robles-Molina, and María del Pino Palacios-Díaz. 2012. "Screening of Emerging Contaminants and Priority Substances (2008/105/EC) in Reclaimed Water for Irrigation and Groundwater in a Volcanic Aquifer (Gran Canaria, Canary Islands, Spain)." *Science of the Total Environment* 433: 538–46. <https://doi.org/10.1016/j.scitotenv.2012.06.031>.
- "European Commission." 2000.
- Farré, Marinel la, Sandra Pérez, Lina Kantiani, and Damià Barceló. 2008. "Fate and Toxicity of Emerging Pollutants, Their Metabolites and Transformation Products in the Aquatic Environment." *TrAC - Trends in Analytical Chemistry* 27 (11): 991–1007. <https://doi.org/10.1016/j.trac.2008.09.010>.
- Fent, Karl, Anna A. Weston, and Daniel Caminada. 2006. "Ecotoxicology of Human Pharmaceuticals." *Aquatic Toxicology* 76 (2): 122–59. <https://doi.org/10.1016/j.aquatox.2005.09.009>.
- Ferrando-Climent, Laura, Neus Collado, Gianluigi Buttiglieri, Meritxell Gros, Ignasi Rodriguez-Roda, Sara Rodriguez-Mozaz, and Damià Barceló. 2012. "Comprehensive Study of Ibuprofen and Its Metabolites in Activated Sludge Batch Experiments and Aquatic Environment." *Science of the Total Environment* 438: 404–13. <https://doi.org/10.1016/j.scitotenv.2012.08.073>.

- Fitch, M. W. 2014. "Constructed Wetlands." In *Comprehensive Water Quality and Purification*, 3:268–95. Elsevier. <https://doi.org/10.1016/B978-0-12-382182-9.00053-0>.
- Frascaroli, Gabriele, Deborah Reid, Colin Hunter, Joanne Roberts, Karin Helwig, Janice Spencer, and Ania Escudero. 2021. "Pharmaceuticals in Wastewater Treatment Plants: A Systematic Review on the Substances of Greatest Concern Responsible for the Development of Antimicrobial Resistance." *Applied Sciences (Switzerland)* 11 (15). <https://doi.org/10.3390/app11156670>.
- Gandiglio, Marta, Andrea Lanzini, Alicia Soto, Pierluigi Leone, and Massimo Santarelli. 2017. "Enhancing the Energy Efficiency of Wastewater Treatment Plants through Co-Digestion and Fuel Cell Systems." *Frontiers in Environmental Science* 5 (October): 1–21. <https://doi.org/10.3389/fenvs.2017.00070>.
- Gavrilescu, Maria, Kateřina Demnerová, Jens Aamand, Spiros Agathos, and Fabio Fava. 2015. "Emerging Pollutants in the Environment: Present and Future Challenges in Biomonitoring, Ecological Risks and Bioremediation." *New Biotechnology* 32 (1): 147–56. <https://doi.org/10.1016/j.nbt.2014.01.001>.
- Geissen, Violette, Hans Mol, Erwin Klumpp, Günter Umlauf, Marti Nadal, Martine van der Ploeg, Sjoerd E.A.T.M. van de Zee, and Coen J. Ritsema. 2015. "Emerging Pollutants in the Environment: A Challenge for Water Resource Management." *International Soil and Water Conservation Research* 3 (1): 57–65. <https://doi.org/10.1016/j.iswcr.2015.03.002>.
- Green, Michal, Eran Friedler, Yuri Ruskol, and Iris Safrai. 1997. "Investigation of Alternative Method for Nitrification in Constructed Wetlands." *Water Science and Technology* 35 (5): 63–70. [https://doi.org/10.1016/S0273-1223\(97\)00053-X](https://doi.org/10.1016/S0273-1223(97)00053-X).
- Guignard, Maïté S., Andrew R. Leitch, Claudia Acquisti, Christophe Eizaguirre, James J. Elser, Dag O. Hessen, Punidan D. Jeyasingh, et al. 2017. "Impacts of Nitrogen and Phosphorus: From Genomes to Natural Ecosystems and Agriculture." *Frontiers in Ecology and Evolution* 5 (JUL). <https://doi.org/10.3389/fevo.2017.00070>.
- Hidayah, Euis Nurul, Yung-Chen Chou, and Hsuan-Hsien Yeh. 2018. "Characterization and Removal of Natural Organic Matter from Slow Sand Filter Effluent Followed by Alum Coagulation." *Applied Water Science* 8 (1). <https://doi.org/10.1007/s13201-018-0671-4>.
- Huguenot, David, Paul Bois, Karine Jézéquel, Jean Yves Cornu, and Thierry Lebeau. 2010. "Selection of Low Cost Materials for the Sorption of Copper and Herbicides as Single or Mixed Compounds in Increasing Complexity Matrices." *Journal of Hazardous Materials* 182 (1–3): 18–26. <https://doi.org/10.1016/j.jhazmat.2010.05.062>.
- IISD. 2003. "Forum Bulletin A Daily Report of the 3rd World Water Forum and Ministerial Conference." *Forum Final* 82 (8): 1–14. <http://www.iisd.ca/SD/3WWF/>.
- INWRDAM, AMMAN, JORDAN. 2016. "Constructed Wetland Manual." *Inter*

- Jamieson, T. S., G. W. Stratton, R. Gordon, and A. Madani. 2002. "Phosphorus Adsorption Characteristics of a Constructed Wetland Soil Receiving Dairy Farm Wastewater." *Canadian Journal of Soil Science* 82 (1): 97–104. <https://doi.org/10.4141/S01-042>.
- Jean-Christophe Vié, Craig Hilton-Taylor and Simon N. Stuart. 2010. *State of the World's Oceans. Marine Ecology*. Vol. 31. <https://doi.org/10.1111/j.1439-0485.2010.00364.x>.
- Jensen, Frank B. 2003. "Comparative Biochemistry and Physiology Part Nitrite Disrupts Multiple Physiological Functions in Aquatic Animals" 135: 9–24.
- Jia, Yanyan, Samir Kumar Khanal, Linwan Yin, Lianpeng Sun, and Hui Lu. 2021. "Influence of Ibuprofen and Its Biotransformation Products on Different Biological Sludge Systems and Ecosystem." *Environment International* 146: 106265. <https://doi.org/10.1016/j.envint.2020.106265>.
- Jiménez-Silva, Vanessa A., Fortunata Santoyo-Tepole, Nora Ruiz-Ordaz, and Juvencio Galíndez-Mayer. 2019. "Study of the Ibuprofen Impact on Wastewater Treatment Mini-Plants with Bioaugmented Sludge." *Process Safety and Environmental Protection* 123: 140–49. <https://doi.org/10.1016/j.psep.2018.08.006>.
- Kapelewska, Justyna, Urszula Kotowska, Joanna Karpińska, Diana Kowalczyk, Agnieszka Arciszewska, and Anna Świrydo. 2018. "Occurrence, Removal, Mass Loading and Environmental Risk Assessment of Emerging Organic Contaminants in Leachates, Groundwaters and Wastewaters." *Microchemical Journal* 137: 292–301. <https://doi.org/10.1016/j.microc.2017.11.008>.
- Karen Bush. 1997. "Antimicrobial Agents." *Seminars in Dialysis* 23 (5): 472–74. <https://doi.org/10.1111/j.1525-139X.2010.00774.x>.
- Khudr, Mouhammad Shadi, Yassin Mohamed Elhassan Ibrahim, Arthur Garforth, and Abdullatif Alfutimie. 2021. "On Copper Removal from Aquatic Media Using Simultaneous and Sequential Iron-Perlite Composites." *Journal of Water Process Engineering* 40 (July 2020): 101842. <https://doi.org/10.1016/j.jwpe.2020.101842>.
- Kolpin, Dana W., Edward T. Furlong, Michael T. Meyer, E. Michael Thurman, Steven D. Zaugg, Larry B. Barber, and Herbert T. Buxton. 2002. "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance." *Environmental Science and Technology* 36 (6): 1202–11. <https://doi.org/10.1021/es011055j>.
- Koottatep, Thammarat, and Chongrak Polprasert. 1997. "Role of Plant Uptake on Nitrogen Removal in Constructed Wetlands Located in the Tropics." *Water Science and Technology* 36 (12): 1–8. [https://doi.org/10.1016/S0273-1223\(97\)00725-7](https://doi.org/10.1016/S0273-1223(97)00725-7).
- Kristiansson, Erik, Jerker Fick, Anders Janzon, Roman Grabic, Carolin Rutgersson, Birgitta Weijdegård, Hanna Söderström, and D. G. Joakim Larsson. 2011. "Pyrosequencing of Antibiotic-Contaminated River Sediments Reveals High Levels of Resistance and Gene Transfer Elements." *PLoS ONE* 6 (2).

<https://doi.org/10.1371/journal.pone.0017038>.

- Kunz, Petra Y., and Karl Fent. 2006. "Estrogenic Activity of UV Filter Mixtures." *Toxicology and Applied Pharmacology* 217 (1): 86–99. <https://doi.org/10.1016/j.taap.2006.07.014>.
- Landrigan, Philip J., Richard Fuller, Nereus J.R. Acosta, Olusoji Adeyi, Robert Arnold, Niladri (Nil) Basu, Abdoulaye Bibi Baldé, et al. 2018. "The Lancet Commission on Pollution and Health." *The Lancet* 391 (10119): 462–512. [https://doi.org/10.1016/S0140-6736\(17\)32345-0](https://doi.org/10.1016/S0140-6736(17)32345-0).
- Lee, Chang Gyun, Tim D. Fletcher, and Guangzhi Sun. 2009. "Nitrogen Removal in Constructed Wetland Systems." *Engineering in Life Sciences* 9 (1): 11–22. <https://doi.org/10.1002/elsc.200800049>.
- Lee, Jae Heung. 2013. "An Overview of Phytoremediation as a Potentially Promising Technology for Environmental Pollution Control." *Biotechnology and Bioprocess Engineering* 18 (3): 431–39. <https://doi.org/10.1007/s12257-013-0193-8>.
- León, Víctor M., Inés García, Emilia González, Raquel Samper, Verónica Fernández-González, and Soledad Muniategui-Lorenzo. 2018. "Potential Transfer of Organic Pollutants from Littoral Plastics Debris to the Marine Environment." *Environmental Pollution* 236: 442–53. <https://doi.org/10.1016/j.envpol.2018.01.114>.
- Li, Fengmin, Lun Lu, Xiang Zheng, Huu Hao Ngo, Shuang Liang, Wenshan Guo, and Xiuwen Zhang. 2014. "Enhanced Nitrogen Removal in Constructed Wetlands: Effects of Dissolved Oxygen and Step-Feeding." *Bioresour. Technology* 169: 395–402. <https://doi.org/10.1016/j.biortech.2014.07.004>.
- Li, Luzhen, Chunguang He, Guodong Ji, Wei Zhi, and Lianxi Sheng. 2015. "Nitrogen Removal Pathways in a Tidal Flow Constructed Wetland under Flooded Time Constraints." *Ecological Engineering* 81: 266–71. <https://doi.org/10.1016/j.ecoleng.2015.04.073>.
- Li, Yan, Luyan Zhang, Xianshu Liu, and Jie Ding. 2019. "Ranking and Prioritizing Pharmaceuticals in the Aquatic Environment of China." *Science of the Total Environment* 658: 333–42. <https://doi.org/10.1016/j.scitotenv.2018.12.048>.
- Li, Yifei, Guibing Zhu, Wun Jern Ng, and Soon Keat Tan. 2014. "A Review on Removing Pharmaceutical Contaminants from Wastewater by Constructed Wetlands: Design, Performance and Mechanism." *Science of the Total Environment* 468–469: 908–32. <https://doi.org/10.1016/j.scitotenv.2013.09.018>.
- Liess, Matthias, Sebastian Henz, and Naeem Shahid. 2020. "Modeling the Synergistic Effects of Toxicant Mixtures." *Environmental Sciences Europe* 32 (1). <https://doi.org/10.1186/s12302-020-00394-7>.
- Llamas, Marta, Iñaki Vadillo-Pérez, Lucila Candela, Pablo Jiménez-Gavilán, Carmen Corada-Fernández, and Antonio F. Castro-Gámez. 2020. "Screening and Distribution of Contaminants of Emerging Concern and Regulated Organic Pollutants in the Heavily Modified Guadalhorce River Basin, Southern Spain." *Water (Switzerland)* 12 (11): 1–19. <https://doi.org/10.3390/w12113012>.

- Loganathan, Paripurnanda, Saravanamuthu Vigneswaran, Jaya Kandasamy, and Nanthi S. Bolan. 2014. "Removal and Recovery of Phosphate from Water Using Sorption." *Critical Reviews in Environmental Science and Technology* 44 (8): 847–907. <https://doi.org/10.1080/10643389.2012.741311>.
- Lonappan, Linson, Satinder Kaur Brar, Ratul Kumar Das, Mausam Verma, and Rao Y. Surampalli. 2016. "Diclofenac and Its Transformation Products: Environmental Occurrence and Toxicity - A Review." *Environment International* 96: 127–38. <https://doi.org/10.1016/j.envint.2016.09.014>.
- Lopez, Benjamin, Patrick Ollivier, Anne Togola, Nicole Baran, and Jean Philippe Ghestem. 2015. "Screening of French Groundwater for Regulated and Emerging Contaminants." *Science of the Total Environment* 518–519: 562–73. <https://doi.org/10.1016/j.scitotenv.2015.01.110>.
- LU, Songliu, Hongying HU, Yingxue SUN, and Jia YANG. 2009. "Effect of Carbon Source on the Denitrification in Constructed Wetlands." *Journal of Environmental Sciences* 21 (8): 1036–43. [https://doi.org/10.1016/S1001-0742\(08\)62379-7](https://doi.org/10.1016/S1001-0742(08)62379-7).
- Lyu, Tao. 2016. "Removal of Emerging Organic Pollutants in Constructed Wetlands: Imazalil and Tebuconazole as Model Pesticides," no. August. https://pure.au.dk/ws/files/101709503/PhD_Dissertation_Tao_Lyu.pdf.
- Maheshwari, Dinesh K., Abhinav Aeron, and Meenu Saraf. 2013. *Bacteria in Agrobiolgy: Crop Productivity. Bacteria in Agrobiolgy: Crop Productivity*. <https://doi.org/10.1007/978-3-642-37241-4>.
- Manisalidis, Ioannis, Elisavet Stavropoulou, Agathangelos Stavropoulos, and Eugenia Bezirtzoglou. 2020. "Environmental and Health Impacts of Air Pollution: A Review." *Frontiers in Public Health* 8 (February): 1–13. <https://doi.org/10.3389/fpubh.2020.00014>.
- Markoska, Vesna, Velibor Spalevic, and Rubin Gulaboski. 2018. "A Research on the Influence of Porosity on Perlite Substrate and Its Interaction on Porosity of Two Types of Soil and Peat Substrate." *The Journal "Agriculture and Forestry"* 64 (3): 15–29. <https://doi.org/10.17707/agricultforest.64.3.02>.
- Matamoros, Víctor, Carlos A. Arias, Loc Xuan Nguyen, Victòria Salvadó, and Hans Brix. 2012. "Occurrence and Behavior of Emerging Contaminants in Surface Water and a Restored Wetland." *Chemosphere* 88 (9): 1083–89. <https://doi.org/10.1016/j.chemosphere.2012.04.048>.
- Mazzeo, Dânia Elisa Christofolletti, Carlos Emílio Levy, Dejanira de Franceschi de Angelis, and Maria Aparecida Marin-Morales. 2010. "BTEX Biodegradation by Bacteria from Effluents of Petroleum Refinery." *Science of the Total Environment* 408 (20): 4334–40. <https://doi.org/10.1016/j.scitotenv.2010.07.004>.
- McMichael A. J., Confalonieri U., Brijnath, B. 2012. "2 Environment , Climate Change , Social Factors and the Implications for Controlling Infectious Diseases of Poverty." *World Health Organization*.
- Meagher, Richard B. 2000. "Phytoremediation of Toxic Elemental and Organic Pollutants." *Current Opinion in Plant Biology* 3 (2): 153–62.

[https://doi.org/10.1016/S1369-5266\(99\)00054-0](https://doi.org/10.1016/S1369-5266(99)00054-0).

- Mehinto, Alvine Coralie. 2013. "Impacts of the Human Pharmaceutical Diclofenac in the Aquatic Environment." *Thesis* 53 (9): 1689–99.
- Mehtab Haseena, Muhammad Faheem Malik, Asma Javed, Sidra Arshad, Nayab Asif, Sharon Zulfiqar and Jaweria Hanif. 2017. "Water Pollution and Human Health." *Water, Air, and Soil Pollution* 5 (3): 289–97.
<https://doi.org/10.1007/BF00158344>.
- Memmert, Ulrich, Armin Peither, Roland Burri, Klaus Weber, Thomas Schmidt, John P. Sumpter, and Andreas Hartmann. 2013. "Diclofenac: New Data on Chronic Toxicity and Bioconcentration in Fish." *Environmental Toxicology and Chemistry* 32 (2): 442–52. <https://doi.org/10.1002/etc.2085>.
- Metcalf, Chris D., Shaogang Chu, Colin Judt, Hongxia Li, Ken D. Oakes, Mark R. Servos, and David M. Andrews. 2010. "Antidepressants and Their Metabolites in Municipal Wastewater, and Downstream Exposure in an Urban Watershed." *Environmental Toxicology and Chemistry* 29 (1): 79–89.
<https://doi.org/10.1002/etc.27>.
- Mohebbi Derakhsh, P., A. Mashinchian Moradi, I. Sharifpour, and S. Jamili. 2020. "Toxic Effects of Diclofenac on Gills, Liver and Kidney of *Cyprinus Carpio* (Linnaeus, 1758)." *Iranian Journal of Fisheries Sciences* 19 (2): 735–47.
<https://doi.org/10.22092/ijfs.2018.119517>.
- "Mongabay." n.d. <https://india.mongabay.com/>.
- Montgomery, Tad. 2004. "Constructed Wetlands to Purify Wastewater" 6483 (802): 2004.
- Mylavarapu, Rao. 2008. "Impact of Phosphorus on Water Quality." *SI275*, no. April 2014: 4. <https://edis.ifas.ufl.edu/pdf/SS/SS49000.pdf>.
- Nakada, Norihide, Kentaro Kiri, Hiroyuki Shinohara, Arata Harada, Keisuke Kuroda, Satoshi Takizawa, and Hideshige Takada. 2008. "Evaluation of Pharmaceuticals and Personal Care Products as Water-Soluble Molecular Markers of Sewage." *Environmental Science and Technology* 42 (17): 6347–53.
<https://doi.org/10.1021/es7030856>.
- Nelson, M, F Cattin, Robyn Tredwell, Gove Depuy, Made Suraja, and A Czech. 2007. "Why There Are No Better Systems than Constructed Wetlands to Treat Sewage Water : Advantages , Issues and Challenges." *Society*.
- Norton, Stephen. 2003. "Removal Mechanisms in Constructed Wastewater Wetlands Stephen Norton." *Removal Mechanisms in Constructed Wastewater Wetlands*.
- Nourmohammadi, Davood, Mir Bager Esmaeeli, Hossein Akbarian, and Mohammad Ghasemian. 2013. "Nitrogen Removal in a Full-Scale Domestic Wastewater Treatment Plant with Activated Sludge and Trickling Filter." *Journal of Environmental and Public Health* 2013. <https://doi.org/10.1155/2013/504705>.
- Oaks, J Lindsay, Martin Gilbert, Munir Z Virani, Richard T Watson, Carol U Meteyer, Bruce A Rideout, H L Shivaprasad, et al. 2004. "Diclofenac Residues as The." *Nature* 427 (6975): 630–33.

- OECD. 2000. "OECD 106 Adsorption - Desorption Using a Batch Equilibrium Method." *OECD Guideline for the Testing of Chemicals*, no. January: 1–44. http://www.oecd-ilibrary.org/environment/test-no-106-adsorption-desorption-using-a-batch-equilibrium-method_9789264069602-en.
- Oliveira, Milina de, Alexandre Arruda Atalla, Breno Emanuel Farias Frihling, Priscila Sabioni Cavalheri, Ludovico Migliolo, and Fernando J.C.Magalhães Filho. 2019. "Ibuprofen and Caffeine Removal in Vertical Flow and Free-Floating Macrophyte Constructed Wetlands with *Heliconia Rostrata* and *Eichornia Crassipes*." *Chemical Engineering Journal* 373 (February): 458–67. <https://doi.org/10.1016/j.cej.2019.05.064>.
- Orem, Nicholas R., and Patrick J. Dolph. 2002. "Loss of the Phospholipase C Gene Product Induces Massive Endocytosis of Rhodopsin and Arrestin in *Drosophila* Photoreceptors." *Vision Research* 42 (4): 497–505. [https://doi.org/10.1016/S0042-6989\(01\)00229-2](https://doi.org/10.1016/S0042-6989(01)00229-2).
- Patel, Manvendra, Rahul Kumar, Kamal Kishor, Todd Mlsna, Charles U. Pittman, and Dinesh Mohan. 2019. "Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods." *Chemical Reviews* 119 (6): 3510–3673. <https://doi.org/10.1021/acs.chemrev.8b00299>.
- Perdana, M. C., H. B. Sutanto, and G. Prihatmo. 2018. "Vertical Subsurface Flow (VSSF) Constructed Wetland for Domestic Wastewater Treatment." *IOP Conference Series: Earth and Environmental Science* 148 (1). <https://doi.org/10.1088/1755-1315/148/1/012025>.
- Petrella, Andrea, Danilo Spasiano, Vito Rizzi, Pinalysa Cosma, Marco Race, and Nicoletta De Vietro. 2018. "Lead Ion Sorption by Perlite and Reuse of the Exhausted Material in the Construction Field." *Applied Sciences (Switzerland)* 8 (10): 23–26. <https://doi.org/10.3390/app8101882>.
- Platzer, Christoph. 1999. "Design Recommendations for Subsurface Flow Constructed Wetlands for Nitrification and Denitrification." In *Water Science and Technology*, 40:257–63. No longer published by Elsevier. [https://doi.org/10.1016/S0273-1223\(99\)00420-5](https://doi.org/10.1016/S0273-1223(99)00420-5).
- Pomati, Francesco, Sara Castiglioni, Ettore Zuccato, Roberto Fanelli, Davide Vigetti, Carlo Rossetti, and Davide Calamari. 2006. "Effects of a Complex Mixture of Therapeutic Drugs at Environmental Levels on Human Embryonic Cells." *Environmental Science and Technology* 40 (7): 2442–47. <https://doi.org/10.1021/es051715a>.
- Pomati, Francesco, Chiara Orlandi, Moira Clerici, Fabio Luciani, and Ettore Zuccato. 2008. "Effects and Interactions in an Environmentally Relevant Mixture of Pharmaceuticals." *Toxicological Sciences* 102 (1): 129–37. <https://doi.org/10.1093/toxsci/kfm291>.
- Raharjo, Syafrudin, Suprihatin Suprihatin, Nastiti Siswi Indrasti, and Ety Riani. 2015. "Phytoremediation of Vaname Shrimp (*Litopenaeus Vannamei*) Wastewater Using Vetiver Grass System (*Chrysopogon Zizanioides*, L) in Flow Water Surface-Constructed Wetland." *AACL Bioflux* 8 (5): 796–804.
- Ratnakar, Arpna, Shiv Shankar, Vidya Vihar, Raebareli Road, Uttar Pradesh, Vidya

- Vihar, and Uttar Pradesh. 2016. "An Overview of Biodegradation of Organic Pollutants" 4 (1): 73–91.
- Reis, Eduarda O., Ana Flávia S. Foureaux, Júlia S. Rodrigues, Victor R. Moreira, Yuri A.R. Lebron, Lucilaine V.S. Santos, Miriam C.S. Amaral, and Liséte C. Lange. 2019. "Occurrence, Removal and Seasonal Variation of Pharmaceuticals in Brazilian Drinking Water Treatment Plants." *Environmental Pollution* 250: 773–81. <https://doi.org/10.1016/j.envpol.2019.04.102>.
- Richardson, Susan D., and Susana Y. Kimura. 2020. "Water Analysis: Emerging Contaminants and Current Issues." Review-article. *Analytical Chemistry* 92 (1): 473–505. <https://doi.org/10.1021/acs.analchem.9b05269>.
- Rosset, Morgana, Leticia Weidlich Sfredo, Gelsa Edith Navarro Hidalgo, Oscar W. Perez-Lopez, and Liliana Amaral Féris. 2019. "Adsorbents Derived from Hydrotalcites for the Removal of Diclofenac in Wastewater." *Applied Clay Science* 175 (February): 150–58. <https://doi.org/10.1016/j.clay.2019.04.014>.
- Salvestrini, Stefano, Angelo Fenti, Simeone Chianese, Pasquale Iovino, and Dino Musmarra. 2020. "Diclofenac Sorption from Synthetic Water: Kinetic and Thermodynamic Analysis." *Journal of Environmental Chemical Engineering* 8 (5): 104105. <https://doi.org/10.1016/j.jece.2020.104105>.
- Sathishkumar, Palanivel, Ramakrishnan Anu Alias Meena, Thavamani Palanisami, Veeramuthu Ashokkumar, Thayumanavan Palvannan, and Feng Long Gu. 2020a. "Occurrence, Interactive Effects and Ecological Risk of Diclofenac in Environmental Compartments and Biota - a Review." *Science of the Total Environment* 698: 134057. <https://doi.org/10.1016/j.scitotenv.2019.134057>.
- . 2020b. "Occurrence, Interactive Effects and Ecological Risk of Diclofenac in Environmental Compartments and Biota - a Review." *Science of the Total Environment*. Elsevier B.V. <https://doi.org/10.1016/j.scitotenv.2019.134057>.
- Schmitt, Claudia, Matthias Oetken, Olaf Dittberner, Martin Wagner, and Jörg Oehlmann. 2008. "Endocrine Modulation and Toxic Effects of Two Commonly Used UV Screens on the Aquatic Invertebrates *Potamopyrgus Antipodarum* and *Lumbriculus Variegatus*." *Environmental Pollution* 152 (2): 322–29. <https://doi.org/10.1016/j.envpol.2007.06.031>.
- Sehonova, Pavla, Zdenka Svobodova, Petra Dolezelova, Petra Vosmerova, and Caterina Faggio. 2018. "Effects of Waterborne Antidepressants on Non-Target Animals Living in the Aquatic Environment: A Review." *Science of the Total Environment* 631–632: 789–94. <https://doi.org/10.1016/j.scitotenv.2018.03.076>.
- Seruga, Przemysław, Małgorzata Krzywonos, Justyna Pyzanowska, Agnieszka Urbanowska, Halina Pawlak-Kruczek, and Łukasz Niedźwiecki. 2019. "Removal of Ammonia from the Municipal Waste Treatment Effluents Using Natural Minerals." *Molecules* 24 (20). <https://doi.org/10.3390/molecules24203633>.
- Setyono, S Qomariyah AH Ramelan Sobriyah P. 2016. "Use of Macrophyte Plants, Sand & Gravel Materials in Constructed Wetlands for Greywater Treatment." *Journal of Physics: Conference Series* 755 (1): 0–6. <https://doi.org/10.1088/1742-6596/755/1/011001>.

- Shultz, Susanne, Hem Sagar Baral, Sheonaidh Charman, Andrew A. Cunningham, Devojit Das, G. R. Ghalsasi, Mallikarjun S. Goudar, et al. 2004. "Diclofenac Poisoning Is Widespread in Declining Vulture Populations across the Indian Subcontinent." *Proceedings of the Royal Society B: Biological Sciences* 271 (SUPPL. 6): 458–60. <https://doi.org/10.1098/rsbl.2004.0223>.
- Sleytr, Kirsten, Alexandra Tietz, Günter Langergraber, and Raimund Haberl. 2007. "Investigation of Bacterial Removal during the Filtration Process in Constructed Wetlands." *Science of the Total Environment* 380 (1–3): 173–80. <https://doi.org/10.1016/j.scitotenv.2007.03.001>.
- Smook, T. M., H. Zho, and R. G. Zytner. 2008. "Removal of Ibuprofen from Wastewater: Comparing Biodegradation in Conventional, Membrane Bioreactor, and Biological Nutrient Removal Treatment Systems." *Water Science and Technology* 57 (1): 1–8. <https://doi.org/10.2166/wst.2008.658>.
- Stefanakis, Alexandros, Christos S. Akrotas, and Vassilios A. Tsihrintzis. 2014. "VFCW Types." *Vertical Flow Constructed Wetlands*, 27–38. <https://doi.org/10.1016/b978-0-12-404612-2.00003-9>.
- Stottmeister, U., A. Wießner, P. Kuschik, U. Kappelmeyer, M. Kästner, O. Bederski, R. A. Müller, and H. Moormann. 2003. "Effects of Plants and Microorganisms in Constructed Wetlands for Wastewater Treatment." *Biotechnology Advances* 22 (1–2): 93–117. <https://doi.org/10.1016/j.biotechadv.2003.08.010>.
- Stuart, Marianne, Dan Lapworth, Emily Crane, and Alwyn Hart. 2012. "Review of Risk from Potential Emerging Contaminants in UK Groundwater." *Science of the Total Environment* 416: 1–21. <https://doi.org/10.1016/j.scitotenv.2011.11.072>.
- Tang, Yankui, Maozhong Yin, Weiwei Yang, Huilan Li, Yaxuan Zhong, Lihong Mo, Yan Liang, Xiangmeng Ma, and Xiang Sun. 2019. "Emerging Pollutants in Water Environment: Occurrence, Monitoring, Fate, and Risk Assessment." *Water Environment Research* 91 (10): 984–91. <https://doi.org/10.1002/wer.1163>.
- Tejeda, Allan, Arturo Barrera, and Florentina Zurita. 2017. "Adsorption Capacity of a Volcanic Rock-Used in Constructed Wetlands-For Carbamazepine Removal, and Its Modification with Biofilm Growth." *Water (Switzerland)* 9 (9). <https://doi.org/10.3390/w9090721>.
- Tim aus der Beek, Tim, Frank Andreas Weber, Axel Bergmann, Silke Hickmann, Ina Ebert, Arne Hein, and Anette Küster. 2016. "Pharmaceuticals in the Environment-Global Occurrences and Perspectives." *Environmental Toxicology and Chemistry* 35 (4): 823–35. <https://doi.org/10.1002/etc.3339>.
- Tsihrintzis, Vassilios A. 2017. "The Use of Vertical Flow Constructed Wetlands in Wastewater Treatment." *Water Resources Management* 31 (10): 3245–70. <https://doi.org/10.1007/s11269-017-1710-x>.
- Vasilachi, Ionela Cătălina, Dana Mihaela Asiminicesei, Daniela Ionela Fertu, and Maria Gavrilescu. 2021. "Occurrence and Fate of Emerging Pollutants in Water Environment and Options for Their Removal." *Water* 13 (2): 181. <https://doi.org/10.3390/w13020181>.

- Vymazal, Jan. 2007. "Removal of Nutrients in Various Types of Constructed Wetlands." *Science of the Total Environment* 380 (1–3): 48–65. <https://doi.org/10.1016/j.scitotenv.2006.09.014>.
- . 2010. "Constructed Wetlands for Wastewater Treatment." *Water (Switzerland)* 2 (3): 530–49. <https://doi.org/10.3390/w2030530>.
- . 2020. "Removal of Nutrients in Constructed Wetlands for Wastewater Treatment through Plant Harvesting – Biomass and Load Matter the Most." *Ecological Engineering* 155 (June): 105962. <https://doi.org/10.1016/j.ecoleng.2020.105962>.
- Wang, Mo, Dong Qing Zhang, Jian Wen Dong, and Soon Keat Tan. 2017. "Constructed Wetlands for Wastewater Treatment in Cold Climate — A Review." *Journal of Environmental Sciences (China)* 57: 293–311. <https://doi.org/10.1016/j.jes.2016.12.019>.
- Westerhoff, Benjamin M., David J. Fairbairn, Mark L. Ferrey, Adriana Matilla, Jordan Kunkel, Sarah M. Elliott, Richard L. Kiesling, Dustin Woodruff, and Heiko L. Schoenfuss. 2018. "Effects of Urban Stormwater and Iron-Enhanced Sand Filtration on *Daphnia Magna* and *Pimephales Promelas*." *Environmental Toxicology and Chemistry* 37 (10): 2645–59. <https://doi.org/10.1002/etc.4227>.
- Whitehouse, Christina R., Joseph Boullata, and Linda A. McCauley. 2008. "The Potential Toxicity of Artificial Sweeteners." *AAOHN Journal : Official Journal of the American Association of Occupational Health Nurses* 56 (6): 251–61. <https://doi.org/10.3928/08910162-20080601-02>.
- Xu, Defu, Jianming Xu, Jianjun Wu, and Akmal Muhammad. 2006. "Studies on the Phosphorus Sorption Capacity of Substrates Used in Constructed Wetland Systems." *Chemosphere* 63 (2): 344–52. <https://doi.org/10.1016/j.chemosphere.2005.08.036>.
- Yan, Yijing, and Jingcheng Xu. 2014. "Improving Winter Performance of Constructed Wetlands for Wastewater Treatment in Northern China: A Review." *Wetlands* 34 (2): 243–53. <https://doi.org/10.1007/s13157-013-0444-7>.
- Yang, Yan, Yaqian Zhao, Ranbin Liu, and David Morgan. 2018. "Global Development of Various Emerged Substrates Utilized in Constructed Wetlands." *Bioresour Technol* 261: 441–52. <https://doi.org/10.1016/j.biortech.2018.03.085>.
- Yu Zhang. 2016. "Sorption and Desorption Dynamics of Selected Non-Steroidal Anti-Inflammatory Drugs in Agricultural Systems." *Chemosphere*, no. August.
- Zhang, Y, G W Price, R Jamieson, D Burton, and K Khosravi. 2017. "Chemosphere Sorption and Desorption of Selected Non-Steroidal Anti-inflammatory Drugs in an Agricultural Loam-Textured Soil." *Chemosphere* 174: 628–37. <https://doi.org/10.1016/j.chemosphere.2017.02.027>.
- Zhang, Yang, Tao Lv, Pedro N. Carvalho, Liang Zhang, Carlos A. Arias, Zhanghe Chen, and Hans Brix. 2017. "Ibuprofen and Iohexol Removal in Saturated Constructed Wetland Mesocosms." *Ecological Engineering* 98: 394–402. <https://doi.org/10.1016/j.ecoleng.2016.05.077>.

- Zhang, Yongjun, Sven Uwe Geißen, and Carmen Gal. 2008. "Carbamazepine and Diclofenac: Removal in Wastewater Treatment Plants and Occurrence in Water Bodies." *Chemosphere* 73 (8): 1151–61.
<https://doi.org/10.1016/j.chemosphere.2008.07.086>.
- Zhu, Wen Ling, Li Hua Cui, Ying Ouyang, Cui Fen Long, and Xiao Dan Tang. 2011. "Kinetic Adsorption of Ammonium Nitrogen by Substrate Materials for Constructed Wetlands." *Pedosphere* 21 (4): 454–63.
[https://doi.org/10.1016/S1002-0160\(11\)60147-1](https://doi.org/10.1016/S1002-0160(11)60147-1).