REMOVAL OF SELECTED PHARMACEUTICAL COMPOUNDS THROUGH VERTICAL FLOW CONSTRUCTED WETLAND

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I dedicate this thesis to my beloved parents who stood by my side and supported me wholeheartedly

APPROVAL OF EXAMINATION

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ABSTRACT

The usage of hundreds of synthetic organic compounds has transformed life. This includes pharmaceuticals and personal care products (PPCPs). These compounds are now abundant in water systems due to their widespread use, fast growth and ease of access. Pharmaceutical and PPCPs pollutants are increasingly brought into waterways by wastewater treatment plants. The cost-effectiveness of constructed wetlands (CWs) in removing the chemical pollutants (PPCPs) from wastewater treatment plant (WWTP) effluents has attracted considerable interest. Therefore, the primary objective of this study was to design vertical flow constructed wetlands (VF-CWs) for eliminating specific pharmaceutical chemicals at different concentrations and retention time. This research aimed to examine the effectiveness of VF-CWs in reducing pharmaceutical contaminants. The study utilized Ciprofloxacin, Ibroufen, Paracetamol and Cefixime for the preparation of the synthetic wastewater. Three distinct types of CWs were constructed, first planted with *Typha Australis*, second with beds and third planted with *Water Hyacinth*. A total of 3 batches were run and the sampling of each batch was conducted every 48 hours. The results showed significant reductions with *Typha Australis* in Batch 1, reducing 65.61% COD, and 67.1% BOD. Batch 2 reduced 56.5% COD and 52.3% BOD. In Batch 3, 47.0% COD and 53.4% BOD were reduced. The *Water Hyacinth* system also performed well in all batches. It reduced 44.5% COD and 51.4% BOD in Batch 1. Batch 2 lowered COD to 44.5% and BOD to 51.9%. Batch 3 lowered COD to 44.5% and BOD to 51.4%. With comparatively lower reductions rates across all 3 batches, the Beds system showed satisfactory treatment as well, Batch 1 lowered COD to 58.2% and BOD to 56.6%. In batch 2, COD dropped to 55.6% and BOD to 52.10% whereas in Batch 3 COD dropped to41.5% and BOD to 50.2%. HPLC analysis highlighted the removal of pharmaceutical contaminants in three batches. The Typha Australis system exhibited the best removal rate of 42.8%, Water Hyacinth system 36.3% and conversely, the Beds system exhibited 32.1% in batch 1. The Typha Australis system removed drugs with best removal rates while Water Hyacinth and beds systems also showed considerable results. The first batch had better treatment results than the other two. Further research is needed to improve removal strategies and sustain wetland systems.

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LIST OF ABBREVIATIONS

BOD Biochemical Oxygen Demand

COD Chemical Oxygen Demand

CODCr Chemical Oxygen Demand (Chromatic Method)

DO Dissolved Oxygen

EOCs Emerging Organic Contaminants

EPA Environmental Protection Agency

HPLC High-Performance Liquid Chromatography

HRT Hydraulic Retention Time

NEQS National Environmental Quality Standards

OLR Organic Loading Rate

PhACs Pharmaceutically Active Compounds

PhCs Pharmaceuticals

PPCPS Pharmaceuticals and Personal Care Products

SD Standard Deviation

SOP Standard Operating Procedure

SRT Solid Retention Time

TDS Total Dissolved Solids

TP Total Phosphorus

TSS Total Suspended Solids

VF-CWS Vertical Flow Constructed Wetlands

WWTPs Wastewater Treatment Plan

CHAPTER 1

INTRODUCTION

Lately, there has been an increasing occurrence of various organic chemicals in water bodies and soils, with many of them being newly introduced onto the market and therefore into the environment. These substances are known as emergent organic contaminants (EOCs). They are currently not regulated and do not have consistent preventative and treatment measures in place (Saidulu, et al., 2021). However, they can have negative effects on ecosystems and degrade the quality of water resources. Pharmaceuticals (PhCs) are a type of emerging organic contaminants (EOCs) that are released into waterways and the natural world through multiple routes. These sources include residential wastewater (discharge), release from sewage treatment facilities (WWTPs), waste products from hospitals and pharmaceutical industries, dumps leachate, and livestock excretion, among others (Barbosa, et al., 2016). EOCs can be categorized into diverse classes based on their chemical compositions and intended use. Pharmaceutically active compounds (PhACs) are a widely recognized group of EOCs. The increasing utilization of PhACs is resulting in the long-lasting presence and extended contact of these substances in the environment, potentially impacting the physiological and metabolic activities of living organisms over time (Majumder, et al., 2019). Instances include the long-term adverse effects of ibuprofen on humans and aquatic ecosystems, the toxicity induced by ketoprofen in the aquatic environment, and the toxicity of diclofenac on bacteria and its potential harm to human health and the aquatic ecosystem. Studies have demonstrated that bisphenol A, a chemical commonly used in the chemical industry, can lead to several health conditions including cancer, diabetes, and early onset of sexual development in women, among other effects (Tran, et al., 2018).

The use of thousands of synthetic organic chemicals has totally transformed human life. These encompass pharmaceuticals and personal care consumables (PPCPs). Effective management of these has become an essential element of a thriving consumer society. The rapid increase of these chemicals and their convenient availability have greatly augmented their presence in ecological systems. They have been associated with adverse consequences in the aquatic environment, which remain incompletely understood (Tahiri, et al., 2023). The alarming issue of pharmaceutical chemicals in water resources has developed from their extensive use and continuous discharge into the aquatic environment. Residues of pharmaceuticals (PCs) are commonly found in surface water bodies and other environmental resources. These residues primarily come from various sources, including residential wastewater, effluent from treatment facilities, animal manure, garbage dumps leachate, commercial hazardous waste, hospitals, and research labs (Anastopoulos, et al., 2020). Even little concentrations of PCs can be hazardous to both land and water-based organisms. The presence of residual pharmaceutical compounds (PCs) in the environment is associated with hormonal disruption, acute toxicity, DNA damage, metabolic and growth problems, bioaccumulation, and reduced reproductive capacity in aquatic animals (Aguilar-Pérez, et al., 2020). The elimination of emerging contaminants (ECs) from wastewater sources has emerged as a formidable obstacle for sewage treatment plants due to the limited efficacy of existing treatment methods in removing these compounds. Various advanced techniques, including ozonation, photooxidation, and radiolysis, have been examined for their ability to efficiently eliminate emerging pollutants. However, many of these methods are not environmentally friendly and cost-effective, which makes them unappealing for developers and managers of urban wastewater facilities (Liu, et al., 2021).

Various pharmaceutical chemicals are being found in environmental components and wastewater treatment plants. Due to the widespread presence, alteration, and identification of pharmaceutical-related substances in water samples, both individuals and government bodies are now increasingly worried about the possible origins and environmental impacts of ECs (Narvaez & Jimenez, 2015). This is mostly due to the unrestricted mobility of ECs in water matrices, which is causing significant detrimental impacts on humans, aquatic organisms, and indigenous plant species, even at minimal levels. Various identification and remediation methods have been suggested and utilized to combat a wide range of pharmaceutical-related emerging contaminants (ECs). The beneficial and adverse effects of medicinal substances have been thoroughly examined (Yu, et al., 2020). Due to a significant lack of research in this area, there has been a strong focus on studying the causes and long-lasting presence of pharmaceutical-related ECs, as well as their both immediate and secondary negative effects. Surface water is mostly affected by pharmaceutical-related emerging contaminants (ECs) that originate from wastewater treatment facilities (WWTPs) (Parra-Saldivar, et al., 2021).

The escalating occurrence of pharmaceuticals in wastewater has generated substantial environmental and public health apprehensions. Primarily, the polar properties of pharmaceuticals and personal care products (PPCPs) result in their little release into the atmosphere, causing them to accumulate in aquatic environments. This accumulation in turn creates significant ecological pressure on aquatic life (Kostich, et al., 2014). Nevertheless, many PPCPs possess a high level of volatility and can be identified in both indoor dust and air. Indoor and outdoor air in Chicago were found to have significant levels of siloxane. Multiple studies have found an increase in the concentration of synthetic musk's and preservatives in indoor dust particles (Yang, et al., 2017). PCs' entries can be categorized as either single-source or indirect source pollution. Point source pollution refers to a well-known and identifiable source that arises from different sites. Examples of primary point sources for polluting soil and water ecosystems include wastewater from pharmaceutical companies, medical sewage, sewage-treatment plants, and household septic tanks. However, it is difficult to precisely determine the precise position of NPS due to its origin from extensive regions. Point and nonpoint source (NPS) contamination impact three vital natural environmental zones: soil, water on the surface, and underground water (Khan, et al., 2020). Pharmaceutical contamination of soil and water is also frequent due to sewage sludge discharge. Sewage sludge discharge into soils and freshwater ecosystems is PCs' ultimate diffuse source. Biosolids, commonly known as sewage sludge, are the residual material that remains after the treatment of wastewater. This is the cause for the detection of thousands of pharmaceuticals and personal care products (PPCPs) in wastewater treatment facilities (WWTPs) globally. In addition, PPCPs can also be introduced into the environment through other sources, including as commercial, healthcare, related to agriculture, and waste from homes, hence contributing to their widespread presence in the environment (Felis, et al., 2020).

The groundwater–surface contact is the second primary source of NPS contamination. Through overflow and downhill movement, this junction serves as a passive way of combining surface and groundwater. A study conducted by Nguyen, et al., (2019) suggested that high quantities of several forms of PC present in a freshwater river and canal have the potential to readily pollute the groundwater. Pollution of surface waters in rivers and lakes has been documented due to surface runoff, landfill leaching, and other factors (Nguyen, et al., 2019). Previous studies have clearly shown that specific drugs are not capable of being removed in most wastewater treatment plants. The presence of such PCs resulting from NPS contamination might very seriously endanger the ecology. The persistent chemical pollutants (PPCPs) in the environment present significant environmental hazards to both humans and other ecological organisms (Adeleye, et al., 2022). The human population is exposed to these chemicals not only using prescription and/or unprescribed medications, but also by inhalation or ingestion of persistent pharmaceutical compounds discharged into the environment. Nevertheless, considering the toxicological findings and the presence of trace environmental quantities of PPCPs ranging from ng/L to ug/L, these pollutants are regarded to have little or no immediate hazardous hazards. Although PPCPs are linked to long-term environmental and human health hazards, it is important not to disregard the ongoing distribution of these chemicals in the environment (Bexfield, et al., 2019).

The extensive utilization and continuous discharge of pharmaceutical compounds into waterways have raised questions regarding the pollution of water resources. A substantial number of pharmaceuticals taken to the body is not completely broken down, resulting in the excretion of unmetabolized drugs and/or their byproducts through feces and urine in household wastewater (Sathishkumar, et al., 2020). Multiple investigations have verified that traditional wastewater treatment facilities (WWTPs) are not consistently effective in eliminating these substances, resulting in their extensive occurrence in all aquatic components (water, sediments, biota, microfilms). Approximately 80% of nations worldwide discharge their wastewater directly into the environment without undergoing any form of remediation (Ilyas & Masih., 2017). Environmental concentrations are influenced by several sources of contamination, such as cities, agriculture, animal husbandry, industry, and hospitals. Additionally, hydroclimatic factors, including temperature, rainfall, drought, and season, also have a role in determining these levels. Consequently, the susceptibility of the environment to this degradation has sparked extensive debates over the necessity of implementing measures that facilitate the preservation of environmental sustainability (Kakimoto & Onoda, 2019).

Numerous physical, chemical, and biological processes can alter pharmaceuticals, producing transformation products 50 or metabolites 51 that may be more dangerous or persistent than the original compound. Pharmaceuticals (or their metabolites, transformation products, and APIs) are released into the environment at every stage of their lifecycle, including manufacturing, use, and disposal. 52 Since the environment is complex due to multiple sources and transformation and transfer processes, it is challenging to determine the actual exposure of biota and humans. Additionally, there are still significant knowledge gaps regarding the possible impacts of long-term exposure to low concentrations of pharmaceutical and other chemical mixtures on the environment and human health. (Li et al., 2021).

When pharmaceutical chemicals and their byproducts enter the water environment, even at very low levels, they pose potential dangers to the health of marine organisms and humans (Cunningham, et al., 2006). The detrimental impacts on aquatic ecosystems encompass the breeding of male fish, deterioration of renal, gill, and liver functions in fish, development of resistance to pathogens, and reduction in microbial diversity (Pinto, et al., 2022). When humans are exposed to diclofenac (a medication that relieves pain and reduces inflammation), it has been observed that the liver experiences degeneration and autoimmune conditions, which have harmful effects on human health. Furthermore, the pharmacological mixture (comprised of atenolol, carbamazepine, ciprofloxacin, furosemide, ibuprofen, sulfamethoxazole, etc.) was found to suppress the development of human embryonic cells when injected (Srain, et al., 2021).

WWTPs serve as the primary pathway for pharmaceutical compounds to enter various water bodies. These compounds are not effectively removed during the treatment process since the WWTPs were not specifically designed to eliminate them. Consequently, the chemicals stay unchanged and are released into the soil or neighboring surface water bodies (Mohapatra, et al., 2016). Over the past ten years, there has been a rise in environmental consciousness, and the management of environmental pollution and contamination has become the primary focus of relevant government agencies worldwide. Several novel methodologies have been lately investigated to ascertain the mechanisms for eliminating pharmaceuticals from wastewater. Nevertheless, the high expense of these sophisticated treatment procedures renders the widespread implementation financially unfeasible. Thus, choosing inexpensive alternative technologies for pharmaceutical therapy is quite important, particularly in impoverished areas (Delgado, et al., 2020). Hence, there is significant interest in the utilization of built wetlands, which are cost-effective to construct, operate, and sustain, for the purpose of eliminating pharmaceutical toxicants from wastewater. Wetlands have emerged as a highly efficient remediation technology that has garnered attention from environmentalists for the removal of wastewater pollutants. Natural wetlands, by use of several organic procedures such as biodegradation, sorption, Phyto-stabilization, phytoextraction, and Rhizo-filtration, effectively eliminate pollutants from water supplies (Hassan, et al., 2021).

Constructed wetlands (CWs) are an economical method that can be utilized to eliminate antibiotics and steroids, pharmaceuticals and a wide range of emerging pollutants from wastewater. The CWs system is a remediation technique that utilizes biological mechanisms, including biodegradability, adsorption, volatilization, hydrolysis, and photodegradation, to eliminate contaminants (Verlicchi. & Zambello, 2014). Constructed wetlands (CWs) are frequently employed for the treatment of both domestic and industrial wastewater in order to eliminate contaminants such as nitrogen, phosphorus, and suspended particles. Nevertheless, in recent times, constructed wetlands (CWs) have also been employed for the purpose of treating micropollutants found in wastewater due to their cost-effectiveness in terms of design and operation, as well as their simplicity in terms of repairs, and their ability to achieve high levels of pollutant removal. A recent study claimed a 90% clearance effectiveness of constructed wetlands (CWs) in eliminating antibiotics and steroids. The main processes for removing substances include substrate adsorption, biodegrading, and intake by plants. The type of substrate employed in the CWs system is a crucial aspect that influences its efficacy (Hu, et al., 2021). This is because the substrate determines the environmental conditions within the porous areas where the remediation process takes place. A wide range of substrates, including gravel, sand, clay, marble, calcite, fly ash, vermiculite, slag, and bentonite, were extensively utilized in the constructed wetlands (CWs) system. While there have been limited studies on the impact of various factors on the effectiveness of constructed wetlands (CWs), there is a lack of research specifically examining how different types and sizes of substrates affect the removal of antibiotics, steroids and pharmaceutical compounds (Kamilya, et al., 2023).

World-wide, constructed wetlands have been extensively employed for the purpose of wastewater treatment. For example, CW technology has been employed in Europe sincethe latter part of the previous century. Germany was the pioneering country to adopt CW technology in Europe (Christofilopoulos, et al., 2019). Numerous additional nations, including as the United Kingdom, Austria, Slovenia, Switzerland, and Denmark, have operational CWs. Several African countries, including South Africa, Tanzania, Kenya, and Seychelles have implemented CW technology. The purpose of constructed wetlands is to replicate the natural mechanisms involved in the removal and degradation of pollutants in wastewater (Vymazal, et al., 2021). Various types of polluted wastewater, such as sewage from cities, industrial waste (including hydrocarbon refinery and sour-water treatment wastewater byproducts), agricultural wastewater, rainwater, textile wastewater, landfill leachates, and mine drainage, can be effectively treated by wetlands. The utilization of natural processes in the degradation of pollution by constructed wetlands makes it an ecologically sustainable repair approach with minimal negative environmental consequences (Truu, et al., 2015). CW is specifically engineered to process wastewater from various origins in a manner almost identical to conventional effluent treatment facilities. Nevertheless, the application of built wetlands for the removal of pharmaceutical pollutants in wastewater is a relatively new territory. Thorough knowledge of the removal efficiencies, removal methods, design and environmental impacts, and toxicity risks is necessary to determine the practicality of artificial wetlands in eliminating pharmaceuticals in wastewater. Hence, much more attention is needed to pay for these issues in future research studies (Almuktar, et al., 2018).

In general, synthetic wetlands can be categorized based on several factors including hydrology (surface-flow and subsurface-flow), macrophyte kinds (independent, spontaneous, and immersed), and flow direction (horizontal or vertical). Indeed, there are other categories of artificial wetlands, such as surface flow (SF) wetlands, subsurface flow (SSF) wetlands, and mixed systems that combine both surface and subsurface flow wetlands (Agarry, et al., 2018). Engineered systems known as constructed wetlands (CWs) are specifically created to harness natural processes including wetland plants, soils, and related microorganisms for the purpose of treating wastewater, which includes the elimination of pharmaceuticals (PhCs). Many research have examined several categoriesof manmade wetlands, each with unique attributes and removal efficiencies (Kataki, et al., 2021). Wetlands constructed with a free water surface, known as Free Water Surface Constructed Wetlands (FWSCWs), are specifically engineered to promote processes such as photodegradation caused by the direct contact of water particles to sunlight. Their efficacy is limited to specific categories of photochemical that are susceptible to degradation by light. Vertical Flow Constructed Wetlands (VF characterized by the

horizontal movement of water through the substrate, which facilitates interactions between microbial populations and plant roots. These systems are renowned for their capacity to enhance the mechanisms of biodegradation and adsorption (Vymazal., 2022). Vertical flow constructed wetlands (VFCWs) function by enabling the vertical movement of water through the substrate, therefore improving air and facilitating aerobic biodegradation. The existence of aerobic microbial populations makes them highly efficient in eliminating biodegradable persistent organic contaminants (PhCs). Different types of continuous flow systems (CWs) can exhibit considerable variations in performance depending on design and operating parameters, including hydraulic retention time (HRT), organic loading rate (OLR), and the unique properties of the drugs being processed. This study highlights the significance of the kind of CWs in determining the effectiveness of removing particulate contaminants (PhCs). HFCWs are often the most efficient, followed by VFCWs and FWSCWs. In general, the selection of CW type and its design should be customized to suit the particular pollutants and environmental conditions in order to maximize the effectiveness of removal and reduce ecological hazards. (Parde, et al., 2021).

The primary strategies for removing drugs in built wetlands (CWs) include: The main mechanism involves the decomposition of medicines by microbes through both aerobic and anaerobic mechanisms (Gikas, et al., 2021). Pharmaceuticals attach to soil particles and organic debris, facilitating their extraction from water. Pharmaceuticals are absorbed by certain wetland plants through their roots, which helps to reduce contamination. Exposure to sunlight causes the degradation of specific medications, which improves their elimination. The efficiency of these mechanisms might vary depending on the individual parameters and layout of the manmade wetland, and they typically act in conjunction with one other (Ilyas, et al., 2020).

Constructed wetlands (CWs) are a promising method for removing toxins since they may harness natural processes for remediation. Nevertheless, despite the increasing amount of research conducted on constructed wetlands (CWs), there are still significant gaps in our knowledge regarding their efficacy in eliminating pharmaceuticals from synthetic water that contains medicinal substances (Falahi, et al., 2022). It is crucial to identify while addressing these research gaps in order to optimize the design and operation of CW (constructed wetlands), guarantee their effectiveness, and eventually contribute to enhancing water quality and protecting the environment. Many studies identified several areas that require additional research in order to improve the effectiveness of built wetlands in removing pharmaceuticals (Auvinen, et al., 2017). Further research is required to conduct comprehensive investigations on the effectiveness of removing a broader spectrum of medications, especially those that have received less attention or are newly recognized as pollutants. Although biodegradation is acknowledged as a crucial process for removing substances, additional investigation is required to comprehend the precise bacterial populations engaged and their relationship with different medications. There is a scarcity of long-term studies that evaluate the enduring effectiveness and consistency of created wetlands in eliminating medications over an extended period. Further investigation is required to assess the impact of several environmental factors, such as temperature, pH, and hydraulic loading rates, on the effectiveness of pharmaceutical removal in constructed wetlands (CWs) (Ávila & García, 2015).

Various design and operation variables shape the efficacy of pharmaceutical elimination in built wetlands (CWs). The velocity of water introduction into the wetland has a substantial impact on the duration of interaction between the wastewater and the treatment media, thereby influencing the effectiveness of pharmaceutical contamination removal. Extended retention periods typically facilitate enhanced breakdown and elimination of pollutants by allowing adequate time for biological processes to take place (Matamoros, et al., 2017). Wetland design, encompassing factors like as depth, surface area, and flow pattern, might impact treatment efficacy. For example, the choice between horizontal and vertical flow architectures can result in varying removal efficiencies. Modifying the selection of plant species can impact the absorption of drugs and augment microbial activity, which is essential for the breakdown of these chemicals (Salcedo, et al., 2018). The adsorption capacity and surface area of the substrate are crucial factors in the cleanup procedures, since they have the potential to serve as sites for bacterial colonization and adsorption of pollutants. The overall efficacy of artificial wetlands in remediating pharmaceuticals can be influenced by factors such as feeding tactics (batch vs. continuous) and seasonal fluctuations. Collectively, these parameters dictate the efficacy of artificial wetlands in treating wastewater that contains pharmaceuticals and personal care items (Gorgoglione & Torretta, 2018).

Studying the impact of various substrate constituents and arrangements on the elimination of pharmaceuticals can aid in enhancing the structure of constructed wetlands for improved efficiency. There is a limited comprehension about the destiny and harmfulness of transition products that are created following the breakdown of medications in constructed wetlands (CWs) (Rabello, et al., 2019). There is a lack of research on the combined impacts of constructed wetlands (CWs) and other treatment technologies, such as advanced oxidation processes, to improve the removal of pharmaceuticals. Enhanced risk evaluations and evaluation techniques are necessary for reviewing the environmental consequences of medications that may not be eliminated by CWs. To improve the efficiency and dependability of artificial wetlands as a sustainable method for removing pharmaceuticals from wastewater, it is important to address these deficiencies (Gorito, et al., 2017).

1.1 Problem statement

The growing environmental concerns are due to the presence of various organic pollutants known as emerging contaminants (ECs), which include biologically active substances originating from pharmaceutical companies. The complex composition of active pharmaceutical ingredients (APIs) and other chemicals in pharmaceutical manufacturing effluent presents a significant environmental problem (Khasawneh & Palaniandy, 2021). Traditional treatment approaches face difficulties in efficiently eliminating these chemicals, resulting in environmental damage and failure to comply with requirements. Effective resolution of this problem necessitates pioneering investigation into sophisticated, economically viable, and environmentally friendly treatment methods to effectively eradicate pharmaceutical remnants and safeguard water quality in various industrial operations. Considering the worldwide water shortage, it is imperative to investigate unconventional water sources. Insufficient sanitation and improper disposal of wastewater provide major environmental and public health hazards, underscoring the need of treating and recycling wastewater (Wang, et al., 2023). Pharmaceutical and personal care products (PPCPs) are a growing category of contaminants that is frequently introduced into water bodies via wastewater treatment plants (WWTPs). The cost-effectiveness of constructed wetlands (CWs) in removing process and chemical pollutants (PPCPs) from wastewater treatment plant (WWTP) effluents has attracted considerable interest. Hence, the primary objective of this study is to examine the environmental advantages of vertical flow constructed wetlands (CWs) in eliminating specific pharmaceutical chemicals. This research aims to examines the effectiveness of VF-CWs in removing pharmaceutical contaminants, considering some important factors, and potential areas for future study. The goal is to achieve high removal efficiencies and get the anticipated results.

1.2 Research objectives

Our study aims to investigate the following key objectives:

- To design a vertical flow wetland system for treatment of selected pharmaceutical compounds
- To assess the removal of selected pharmaceutical compounds at different concentrations and different time durations.

1.3 Significance of the study

Conventional wastewater treatment plants (WWTPs) are unable to fully eliminate all incoming contaminants. Consequently, in recent years, research has commenced to explore the feasibility of eliminating these novel compounds using alternative wastewater treatment systems (Torrijos, et al., 2016). Constructed wetlands (CWs) are environmentally benign solutions that are considered part of nature-based solutions. They have demonstrated excellent effectiveness in terms of traditional treatment. Hybrid constructed wetlands (CWs) are CWs that integrate two or more types of CWs in a sequential manner. Typically, these hybrid systems integrate horizontal (HF) and vertical (VF) subsurface flow constructed wetlands (CWs), and sometimes surface flow (SF) units (Dhangar & Kumar, 2020).

Vertical Flow Constructed Wetlands (VF-CWs) and other CWs are important for pharmaceutical elimination from synthetic water for various reasons. CWs, notably VF-CWs, are cheaper to build, operate, and maintain than conventional wastewater treatment methods. This makes them a good wastewater treatment solution in resource-constrained settings (Verlicchi & Zambello, 2014). Wastewater is treated by CWs using soil, plants, and microbes. This ecological strategy reduces pollution, encourages biodiversity, and improves the environment. CWs can eliminate new contaminants like pharmaceuticals and personal care chemicals, according to studies. Vegetation and wetland design can help degrade and absorb these toxins. VF-CWs can handle varied wastewaters and climates. Their versatility allows them to be used in urban and rural environments (Sánchez, et al., 2022). By eliminating pharmaceuticals and other contaminants, CWs improve distributing water quality, preserving aquatic ecosystems and human health. Continuous study on CW performance, including design factors and operational tactics, improves pollutant removal. Our study optimizes CW design and operation for specific pollutants, including medicines. Overall, VF-CWs and CWs are a potential and sustainable solution to pharmaceutical pollution in water bodies, offering an effective alternative to standard treatment procedures (Zhang, et al., 2023). Moreover, CWs have the potential to enhance the retrieval of nutrients and other vital elements from wastewater, therefore fostering a circular economy across water management. Further investigation of CWs deepens knowledge of their structure, functioning, and efficacy, resulting in enhanced systems and procedures for pharmacological elimination. Overall, this study is essential for the development of sustainable solutions to the fate of pharmaceuticals (PCs) in waterbodies, the resulting water contamination, the preservation of ecosystems especially the aquatic ecosystems, and the assurance of public health. In summary, the utilization of vertical flow-built wetlands shows great potential in treating pharmaceutical-contaminated wastewater, providing an eco-friendly and effective remedy for emerging pollutants in water sources. (Ravikumar, et al., 2022).

CHAPTER 2

LITERATURE REVIEW

2.1 Literature review

Constructed wetlands (CWs) are an economical nature-based approach for wastewater treatment. Several studies have demonstrated that typical CW designs may effectively eliminate pharmaceutically active chemicals (PhACs) from wastewater mostly by sorption, biodegradation, and photodegradation mechanisms. However, current study has suggested new layouts of CW (constructed wetlands) to enhance the removal of PhACs (pharmaceuticals and personal care products). A study by Escola & Matamoros, (2020) examined previous studies on three innovative techniques for constructed wetlands (CWs): alteration of the infill material used in CWs, augmentation of biodegradation processes, and integrating CWs with advanced wastewater treatment technology. Waste-to-product filling materials, such as biochar and cork, can be utilized to improve the adsorption abilities of constructed wetlands (CWs). Alternatively, the process of biodegradation can be enhanced by integrating bacterial fuel cells, induced airflow, or biological enhancement technology into constructed wetlands (CWs). Ultimately, the utilization of CWs in conjunction with advanced wastewater treatments can provide a broader array of biodegradation routes for pharmaceuticals and a decrease in the creation of byproducts. Future study on constructed wetlands (CWs) should incorporate the surveillance of treatment processes (TPs), an increased range of pharmaceutical active compounds (PhACs), assessment of ecotoxicological impacts, and investigation of antibiotic resistance. Furthermore, a significant drawback of CW technology remains the need for a large surface area, which could potentially be addressed by future research that integrates innovative CW solutions (Escolà & Matamoros, 2020).

Ilyas & Eric, (2019) assessed the impact of structure, functioning, and physicochemical factors of built wetlands (CWs) on the elimination of pharmaceuticals (PhCs). The correlational study demonstrated that the effectiveness of constructed wetlands (CWs) is influenced by various design and operational variables such as area, depth, hydraulic transferring rate, organic compounds loading rate, and water retention time, as well as physical chemical characteristics like dissolved oxygen, temperature, and ph. Additionally, the analysis indicates that approximately 50% of the tested PhCs exhibit a noteworthy relationship with two or more factors in terms of their removal efficiency. Plants had a substantial role in removing certain PhCs by both direct absorption and by improving the mechanism of biological decomposition. The utilization of a substrate that had a significant adsorption ability, abundant organic matter, and a large surface area improved the elimination of certain PhCs (codeine, clarithromycin, erythromycin, ofloxacin, oxytetracycline, carbamazepine, and atenolol) in constructed wetlands through adsorption/sorption processes, which are the main pathways for their removal. Seasonal variations were observed in the elimination of most of the examined PhCs. However, statistically significant differences were found in the elimination of naproxen, salicylic acid, caffeine, and sulfadiazine. To effectively remove PhCs, it is necessary to create constructedwetlands (CWs) that promote biodegradation and other processes. The layout should also optimize design and operational aspects, as well as the physical and chemical parameters (Ilyas & Eric, 2019).

A study conducted by Ilyas & Hullebusch, (2020) did a thorough and evaluative analysis of four different types of constructed wetlands (CWs): free water surface CW (FWSCW), vertical flow CW (VFCW), horizontal flow CW (HFCW), and hybrid CW (HCW). The purpose of the study was to determine the effectiveness of these CWs in removing 29 pharmaceuticals (PhCs) and 19 transformation products (TPs). The analysis was based on an extensive data set compiled from 247 CWs that were reported in 63 peer-reviewed journal papers. Biological degradation, particularly aerobic biodegradation, is the primary method of removing 16 out of 29 PhCs. Other processes such as adsorption/sorption, intake by plants, and photodegradation also play a role in their removal. The healthcare worker (HCW) demonstrated superior performance, followed by the vaccine for children (VFCW), the high-frequency continuous wave (HFCW), and the fixed wireless system continuous wave (FWSCW). The improved removal in healthcare wastewater (HCW) may be attributed to the presence of both aerobic and anaerobic conditions, as well as a longer hydraulic retention time. The use of multiple compartments improves the degradation of pharmaceutical compounds (PhCs) such as diclofenac, acetaminophen, sulfamethoxazole, sulphapyridine, trimethoprim, and atenolol. These compounds are extracted through both biological mechanisms and adsorption/sorption methods. Artificial aeration increased dissolved oxygen, which helped remove PhCs, which breakdown aerobically. Moreover, the enhanced efficiency of aerated constructed wetlands (CWs) may be attributed to the creation of diverse microbial environments with distinct physical and chemical conditions (aerobic and anaerobic). This enables the utilization of both aerobic and anaerobic pathways of metabolism for the elimination of PhCs. The elimination of certain PhCs occurs through the creation of their transformation products (TPs), and the characteristics of these TPs (whether they are persistent or biodegradable/non-biodegradable) significantly influence the method of their elimination (Ilyas & Hullebusch, 2020)

A study conducted by Venditti, et al., (2022) evaluated the effectiveness of six distinctive substrates in Vertical Flow Constructed Wetlands (VFCWs) for removing 27 newly identified pollutants from municipal wastewater. The substrates included sand filled with activated or non-activated biochar or zeolite in various ratios. The VFCWs had been planted with Phragmites australis and Iris pseudacorus. The laboratory study, conducted over a period of 357 days, involved regulated environments where constant amounts of pollutants were added to artificial wastewater. The results demonstrated that Vertical Flow Constructed Wetlands (VFCWs) can achieve exceptional removal of both macro- and micropollutants, resulting in high-quality effluent. Because the removal efficiency was over 90% in the majority of cases, noticeable disparities amid the substrates were not discernible. Substances with intermediate removal, such as AMPA, were shown to be strongly influenced by the kind of substrate. The highest amount of the active component immobilized per unit of substrate has been measured as 0.77 µg of AMPAper gram of 30% biochar mixed with sand. Three highly promising substrates were chosen from the research setting for testing under real settings, including fluctuations in content and fluctuating temperature. Consequently, VFCWs with a mixture of 15% activated charcoal and sand shown efficacy in eliminating 18 developing pollutants and meeting national discharge regulations for 4 specific chemicals (Venditti, et al., 2022)

Gikas, et al., (2021) evaluated the effectiveness of a built wetland (CW) in removing six newly identified contaminants (EPs) from wastewater generated by the campus of a university. The EPs that were considered include: diethyl phthalate (DEP), di-isobutyl phthalate (DIBP), di-n-octyl phthalate (DNOP), bis(2-ehtylxexyl) phthalate (DEHP), tris(1-chloro-2-propyl) phosphorus (TCPP), and caffeine (CAF). Six pilot-scale constructed wetlands (CWs) were utilized, consisting of three straight underground flow (HSF) systems and three vertically flowing (VF) systems, each with distinct design arrangements. The HSF systems included two types of plants and one unplanted system, while the VF systems employed two distinct wastewater consuming methods. Additionally, the HSF systems were operated at two distinctive hydraulic retention times (HRT). The results indicated that the mean removal rates in the three horizontal subsurface flow constructed wetlands (HSF-CWs) varied between 84.3% and 99.9%, 79.0% and 95.7%, 91.4% and 99.7%, 72.2% and 81.0%, 99.1% and 99.6%, and 99.3% and 99.6% for the pollutants DEP, DIBP, DNOP, DEHP, TCPP, and CAF, respectively. The mean removal efficiencies for DEP, DIBP, DNOP, DEHP, TCPP, and CAF in the three VF-CWs were 98.6-99.4%, 63.6-98.0%, 96.6-97.8%, 73.6-94.5%, 99.3-99.5%, and 94.4-96.3%, respectively. The study suggests that the primary methods of removing the target emerging pollutants (EPs) in constructed wetlands (CWs) were biological degradation and sorption onto substrate (Gikas, et al., 2021).

Zhang, et al., (2011) conducted research on the effectiveness of tropical horizontal subsurface constructed wetlands (HSSF CWs) featuring the plant Typha angustifolia in removing four commonly used medications—carbamazepine, diclofenac, ibuprofen, and naproxen—within a timeframe of 2 to 4 days. Ibuprofen and naproxen are both medications that have poor solubility in water. After being in beds with plants for four days, the removal rates were significantly higher, reaching 80% for ibuprofen and 91% for naproxen. In comparison, the beds without plants only removed 60% of ibuprofen and 52% of naproxen. This difference was significant. These medications were extracted from synthetic wastewater through the action of plants. The higher levels of oxygen near plant roots likely played a significant role, but other factors in that vicinity, aside from the elevated air levels, also appeared to have an impact. The system struggled to effectively eliminate carbamazepine and diclofenac, both of which are difficult to remove from water. The explanation for this is that they do not dissolve well in water. These compounds were eliminated because they adhered to the organic surfaces available. This explains why the effectiveness of their removal from the planted beds was similar to that from the unplanted beds. The effectiveness of the medicine clearance did not significantly differ between the 2-day and 4-day marks. In this research, the wetlands demonstrated a high efficiency in eliminating pollutants within a timeframe of only 2 to 4 days. This suggests that using such a constructed wetland system could be a practical option in tropical areas, as it needs less land to clean regular waste and some pharmaceutical chemicals from wastewater (Zhang, et al., 2011).

Since pharmaceuticals are not entirely broken down and because of their enduring nature and environmental mobility, their fate during the treatment of effluents is a significant source of concern. In fact, even at low concentrations of thirty, they pose a threat to human health and aquatic life. Vargas, et al., (2021) constructed a wetland wastewater treatment plant (WWTP), where fourteen (31) pharmaceuticals were monitored and evaluated in 32 influent and effluent samples. The investigation focused on essential water quality metrics, assessing the extent of pharmaceutical elimination, their ability to accumulate in organisms, and the impact of wastewater treatment plants (WWTP) on these compounds. For the experiments, the study utilized tools known as Polar Organic Chemical Integrative Samplers (POCIS) along with biofilms. The 35 drug compounds were measured using a high-tech method that combines liquid chromatography with mass spectrometry. The sampling took place in winter (July 2018) and summer (January 2019). The examination of the 37 wastewater treatment plants successfully filtered out solid debris, specific chemicals, and organic substances from residential waste. They managed to eliminate a significant amount of water pollution, but their ability to remove drugs remains limited. It was identified that biofilms are associated with 40 pharmaceuticals and are thought to assist in eliminating them from water sources. Antibiotics have been identified as highly detrimental to marine life, according to various reports (Vargas, et al., 2021).

The performance of constructed wetlands (CWs) in removing pharmaceuticals and pollutants is typically evaluated by means of chemical analyses. An investigation analyzed the effectiveness of different strategies for reducing pharmaceutical pollutants (PhACs), toxic effects, and antibiotic resistance genes (ARGs) in three constructed wetlands (CWs) that process wastewater from treatment plants. They conducted chemical experiments, toxicity assessments, and molecular examinations to achieve their goal. Initially, 17 pharmaceutical chemicals were tested. Out of those, 14 were found, and seven had amounts greater than 0.1 micrograms per liter. The treatment systems examined successfully eliminated some specific pharmaceutical chemicals. However, on average, about 50% of all pharmaceutical chemicals were removed in the vertical subsurface flow treatment system (VSF-CW) which had a lower water flow rate. In comparison, the other two systems with open water surfaces (SF-CWs) exhibited minimal removal. Subsequently, the harmfulness of the wastewater samples with a range of different tests was assessed. While the constructed wetlands (CWs) diminished the overall impact of estrogen, the detrimental effects on the nervous system from the wastewater samples remained unchanged after passing through both CWs examined. The VSF-CW, along with one of the SF-CW, effectively eliminated an integrase gene and three antibiotic resistance genes that were tested. The elevated ARG levels in the remaining SF-CWs, combined with the increase in total bacterial counts in all CWs, could be associated with the expansion of resistant bacteria. Ultimately, the research concluded that the potency of pharmaceutical chemicals is largely associated with their levels of toxicity. Furthermore, decreased removal of organic matter and nutrients appears to correlate with a reduced elimination of pharmaceutical chemicals (PhACs). While ARGs are connected to organic materials, nutrients, certain medications, and the integrase gene, they are not related to individual antibiotics. The failure to eliminate pharmaceutical and personal care products (PhACs) along with their detrimental impacts and antibiotic resistance genes (ARGs) indicates a need for enhancements in the design of constructed wetlands for more effective treatment (He, et al., 2018).

The escalating occurrence of emerging organic pollutants (EOCs) in nature is necessitating the advancement of technology to efficiently eliminate them. Consequently, a comprehensive examination of existing literature was conducted by Sánchez, et al., (2022) to study the behavior of EOCs (Ecological Oxygen Concentrators) during the treatment of urban wastewater. This analysis focused on both large treatment systems and specifically on built wetlands (CWs). The work examined the behavior of electron-withdrawing catalysts (EOCs) in anaerobic digesters (ADs) and sophisticated oxidation techniques, namely in TiO2-based photocatalysis. These procedures are being suggested as effective pre- and post-treatments for conjunction with cyclic voltammetry (CW). The following ten chemicals were examined: acetaminophen (ACE), ofloxacin (OFL), caffeine (CAF), carbamazepine (CBZ), ketoprofen (KET), ibuprofen (IBU), diclofenac (DCL), Clofibric acid (ACB), bisphenol A (BPA), and sotalol (SOT). The physicochemical and biological characteristics of the chosen EOCs generally determine their breakdown processes. In anaerobic and aerobic treatment systems, the primary removal processes are sorption and degradation by bacteria. The integration of anaerobic and aerobic conditions enhances the elimination effectiveness of endoplasmic trophic compounds (EOCs). Nevertheless, distinct pollutants are resistant to removal. In this context, when combined with CWs, TiO2-based photocatalysis shows great potential as a post-treatment method for the efficient elimination of EOC from wastewater (Sánchez, et al., 2022).

The presence of personal care goods and pharmaceuticals (PPCPs) in wastewater from municipalities has raised significant concerns over their potential effects on both people and the natural environment. Constructed wetlands are widely acknowledged as a cost-effective and environmentally friendly method for removing pharmaceuticals and personal care products (PPCPs) from municipal wastewater. Bayati, et al., (2021) assessed the efficacy of a fully built wetlands treatment system (CCWTs) in eliminating 36 pharmaceuticals and personal care products (PPCPs). The mass of pharmaceuticals and personal care products (PPCPs) released by the wastewater treatment plant into the centralized collection and wastewater treatment system (CCWTs) was determined. The effectiveness of removing PPCPs was assessed by considering their physical and chemical characteristics, including the octanol-water separation coefficient, molecular mass, and acid dissociation constant. The CCWTs exhibit high efficacy in eliminating azithromycin, sertraline, tolfenamic acid, and diphenhydramine, with a removal efficiency of over 88%. The rates of elimination of pharmaceuticals and personal care products (PPCPs) in conventional wastewater treatment plants (CCWTs) vary significantly, ranging from 4.7% to 96.7% for antibiotics, 5% to 86% for depression medication and antiseizure drugs, 3.5% to 88% for nonsteroidal anti-inflammatory drugs (NSAIDs), 29% to 77% for β-blockers and cholesterol-lowering drugs, and 5.5% to 94% for other types of PPCPs. These variations are influenced by the physical and chemical properties of the molecules.

Furthermore, the environmental risk assessment revealed that most of the pharmaceuticals and personal care products (except sulfamethoxazole) in the wastewater treatment plant's effluent posed a little risk to aquatic life (risk quotient, RQ ≤ 0.1) because to the effectiveness of the constructed wetland treatment systems (CCWTs). The toxicity index ratings were determined by integrating the expected and known toxicological hazard data using the Toxicological Prioritization Index method (Bayati, et al., 2021).

Natural and built wetlands are increasingly being studied for their potential in wastewater treatment. Although the process of eliminating nutrients in wetlands has been thoroughly studied, documentation on the breakdown of pharmaceuticals and personal care products (PPCPs) has only lately begun to surface. Permanent particulate carbon pollutants (PPCPs) are extensively found in urban wastewaters and can be partially eliminated by the use of artificial wetlands. The medium-term (3-5 years) performance of these solutions in terms of PPCP elimination remains uncertain. Özengin & Elmacı, (2016) assessed the effectiveness of a laboratory-scale artificial wetland composed of Leca and planted with Phragmites australis (Cav.) Trin. Ex. Steudel in treating an aqueous solution containing carbamazepine, ibuprofen, and sulfadiazine. Two pilot-scale built wetlands (CW) were run simultaneously. One CW served as an exploratory unit, with a placed reactor containing P. australis, while the second CW served as a control unit, with a neglected reactor containing Leca organisms. The carbamazepine, ibuprofen, sulfadiazine, and tissue samples (Leca, P. australis body and P. australis leaf) were subjected to pretreatment and analysis using an HPLC instrument. The elimination rates for carbamazepine, ibuprofen, and sulfadiazine in the planted and unplanted units were 89.23% and 95.94%, 89.50% and 94.73%, and 67.20% and 93.68%, respectively. The Leca bed facilitated a highly effective extraction. In the unplanted reactors, Leca has a robust sorption capability for these drugs, with removal rates ranging from 93.68% to 94.94%. The significance of sorption processes in providing effective wastewater treatment, especially in eliminating organic substances that are resistive to biodegradation, is noteworthy. In this context, the constituents of a support matrix may assume a crucial role. The findings from this study demonstrate that a manufactured wetland using Leca as a medium and populated with P. australis is efficient in treating sewage that is polluted with carbamazepine, ibuprofen, and sulfadiazine. (Özengin & Elmacı, Removal of Pharmaceutical Products in a Constructed Wetland., 2016)

Given the global difficulties of water scarcity, it is imperative to consider unconventional water resources to meet the rising demand for clean freshwater. Inadequate sanitation and wastewater disposal infrastructure may lead to environmental and public health issues. Consequently, wastewater treatment and recycling techniques will be essential to ensure enough freshwater supply in the forthcoming decades, as water resources are finite and over 70% of water is utilized for irrigation (Castillo-Valenzuela, et al., 2017). The utilization of treated wastewater for agricultural irrigation holds significant promise, particularly when integrating the recycling of minerals such as nitrogen and phosphorus, which are vital for plant growth. Wetlands have been identified as one of the most effective treatment options for urban wastewater reuse in irrigation, excelling in pollution removal while offering advantages of low maintenance costs and minimal energy requirements. Almuktar, et al., (2018) reviewed in his research and assessed the efficacy of wetlands in wastewater treatment, concluding that it is mostly associated with materials arrangement, substrate characteristics, hydrology, surface loading rate, wastewater feed method, microbial presence, and temperature. created wetlands are highly efficient in eliminating organic matter and suspended particles; nevertheless, nitrogen removal is comparatively limited, however it could be enhanced by employing a combination of diverse forms of created wetlands that comply with irrigation reuse regulations. The elimination of phosphorus is often minimal, unless specialized media with elevated sorption capacity are employed. Eliminating pathogens from wetland effluent to comply with irrigation reuse regulations poses a difficulty until additional lagoons or hybrid wetland systems are implemented (Almuktar, et al., 2018).

The floating treatment wetland (FTW) is an inventive, economical, and ecologically friendly solution for wastewater treatment. The colors in textile effluent deteriorate water quality and adversely affect living organisms. Nawaz, et al., (2020) conducted a research work utilizing floating treatment wetlands (FTWs) planted with *Phragmites australis* and supplemented with bacteria to treat dye-laden synthetic wastewater. Three distinct categories of textile effluent were produced by individually incorporating three different colors into tap water. The FTWs were enhanced with three kinds of bacteria that degrade toxins and promote plant development: *Acinetobacter Juni species, Rhodococcus sp. and Pseudomonas indoloxydans.* The water samples were examined for pH, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD), color, bacterial viability, and heavy metals (Cr, Ni, Mn, Zn, Pb, and Fe). The findings demonstrated that the floating treatment wetlands (FTWs) eliminated contaminants and discoloration from the treated water; yet, the introduction of bacteria alongside plants significantly augmented the remediation efficacy of the floating wetlands (Ladislas, et al., 2015).

In FTWs containing *P. australis* and supplemented with bacterial culture, there was a substantial reduction in pH, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD), and dye color compared to FTWs that were either vegetated or non-vegetated without microbial colonization. Likewise, the FTWs treatment effectively eliminated the heavy metal from the dye-laden effluent, primarily through FTWs injected with bacterial strains. The microbial enhanced vegetation floating treatment wetlands (FTWs) decreased the concentrations of Cu, Ni, Zn, Fe, Mn, and Pb by 75%, 73.3%, 86.9%, 75%, 70%, and 76.7%, respectively, for dye 1. The bacterial treatment of plants for dye 2 resulted in levels of removal of 77.5% for Cu, 73.3% for Ni, 83.3% for Zn, 77.5% for Fe, 66.7% for Mn, and 73.3% for Pb. Similarly, for dye 3, which underwent treatment with plants and colonized bacteria, the metal elimination rates were 77.5% for Cu, 73.3% for Ni, 89.7% for Zn, 81.0% for Fe, 70% for Mn, and 65.5% for Pb. The introduced bacteria exhibited stability in water, as well as in the roots and shoots of the treated plants. The microorganisms diminished dye-induced poisoning and enhanced plant development for each of the dyes. The findings indicated that FTW may be a viable method for the remediation of dye-laden textile wastewater. Additional research is required in this context prior to its commercial application (Nawaz, et al., 2020).

Abed, et al., (2017) conducted research by employing two distinct chemical formulations containing various contaminants used to generate high (HC) and low (LC) concentrations of polluted artificial greywaters (AGW). The cleanup of large-scale floating treatment wetlands (FTW) populated with Phragmites australis (Cav.) Trin. ex Steud. (common reed) has been examined under real weather conditions. The existence or lack of plants, duration of the treatment exposure time, and levels of pollution are the primary variables involved in the layout of the FTW experiment. The emphasis on elimination operations, excluding sedimentation, such as the function of macrophytes in phytoremediation, was accomplished by agitating the treated effluent prior to sampling. Various statistical analyses were employed to evaluate the influence of correlations among each of the operating factors on removing the effectiveness of FTW (Karstens, et al., 2018).

The findings of the study indicated that total suspended solids (TSS) and turbidity levels considerably decreased ($p < 0.05$) in planted wetlands as opposed to unvegetated ones. Vegetation can considerably enhance the biodegradation potential of greywater ($p < 0.05$) by elevating the five-day biochemical oxygen demand (BOD) and reducing the chemical oxygen demand (COD) concentrations. In planted floating treatment wetlands, greater elimination of chemical oxygen demand can be attained while processing high-concentration stormwater compared to low-concentration stormwater. No significant modifications ($p > 0.05$) in BOD removal were observed. Notable improvements ($p < 0.05$) in BOD contents have been observed with prolonged treatment duration, while substantial reductions in COD were observed. Plants in wetlands considerably influenced ($p < 0.05$) the reduction of dissolved oxygen (DO) concentrations in the discharge of both types of greywaters. When organic matter sources are restricted, plants have substantially ($p < 0.05$) improved the nutritional equilibrium by elevating nitrate-nitrogen levels and reducing ortho-phosphate-phosphorus levels in the outflow. Moreover, substantial increases ($p < 0.05$) in ammonia-nitrogen and dissolved oxygen (DO) were noted with prolonged period of contact, whereas total suspended solids (TSS), turbidity, and nitrate- nitrogen concentrations were diminished ($p < 0.05$). Furthermore, yellowing leaves and significantly reduced growth rates were noted in the hydroponic rhizomes of P. australis, potentially resulting from light-induced fluorescein decomposition attributed to the comparatively exposed substrates of the wetlands (Abed, et al., 2017).

CHAPTER 3

MATERIALS AND METHODS

3.1 Selected pharmaceutical compounds of interest

For the preparation of synthetic pharmaceutical wastewater, the study utilized pharmaceutical medicines in order to replicate real-world conditions. *Ciprofloxacin, Ibuprofen, Paracetamol and Cefixime* were utilized for preparation of the synthetic wastewater samples.

3.2 Preparation of synthetic wastewater

Known quantities of pharmaceutical compounds were dissolved in distilled water to create 6, 7 and 8 liters of samples for all three batches. In addition to pharmaceutical compounds, the study also utilized and added different impurities, like organic compounds e.g., phenol, and nutrients like potassium and nitrate. Each sample contained 38mg/l of pharmaceutical compounds at different concentrations and different volumes. Prior to treatment, the following parameters in the synthetic wastewater were examined to compare their results with treated water. These parameters included, total dissolved solids (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD), pH and dissolved oxygen (DO) respectively (Kargol, et al., 2023).

Table 3.1: Pharmaceutical compounds and their respective concentrations for preparing synthetic water

Compounds	**Concentration (mg/L)**	**Concentrations (g/L)**
Paracetamol	7mg/L	0.007g/L
Ciprofloxacin	9mg/L	0.009g/L
Cefixime	11mg/L	0.011g/L
Ibuprofen	(8mg/L	0.008g/L
Phenol	1.6mg/L	0.0016g/L

Potassium Nitrate	1.4 mg/L	0.0014g/L
Total	38 mg/L	0.038g/L

Table 3.2: Pharmaceutical compounds and their respective concentrations for 1st batch of synthetic water

Compounds	**Calculation for 24 liters**	**Concentrations added (g/L)**
Paracetamol	0.007 ×24	0.168
Ciprofloxacin	0.009 × 24	0.216
Cefixime	0.011× 24	0.264
Ibuprofen	0.008 ×24	0.192
Phenol	0.0016 ×24	0.0384
Potassium Nitrate	0.0014 ×24	0.0336

Table 3.3: Pharmaceutical compounds and their respective concentrations for 2nd batch of synthetic water

Compounds	**Calculation for 32 liters**	**Concentrations added (g/L)**
Paracetamol	0.007 ×28	0.196
Ciprofloxacin	0.009 × 28	0.252
Cefixime	0.011× 28	0.308
Ibuprofen	0.008 ×28	0.224
Phenol	0.0016 ×28	0.044
Potassium Nitrate	0.0014 ×28	0.0392

Table 3.4: Pharmaceutical compounds and their respective concentrations for 3rd batch of synthetic water

Compounds	**Calculation for 32 liters**	**Concentrations added (g/L)**
Paracetamol	0.007 ×32	0.224
Ciprofloxacin	0.009 × 32	0.288
Cefixime	0.011× 32	0.352

Ibuprofen	0.008 ×32	0.256
Phenol	0.0016 ×32	0.0512
Potassium Nitrate	0.0014 ×32	0.0448

3.3 Design of vertical flow constructed wetlands (VF-CWs)

A basin or cell composed of reinforced concrete or, more frequently, earth covered with a membrane made of high-density polyethylene (HDPE) for waterproofing were utilized to form the desired constructed wetlands (CWs). Four layers of substrate of different materials such as sand, fine gravel, medium gravel, and charcoal. The charcoal was used as a filter medium. Plants typically *Water Hyacinth and Typha Australis* were used (Hassan, et al., 2021).

The study was carried out in the month of April 2024 in three vertical flow constructed wetlands systems and one controlled system of height (0.6m) and diameter (0.2m). Each vertical flow constructed wetlands (VF-CW) having the same size and measurements. One wetland was made up of four layers, arranged from upper to lower: a layer of coarse gravel (0.15m), a layer of charcoal (0.1m), a layer of fine gravel (0.1m), and a layer of sand (0.1m). One system was planted with *Water Hyacinth,* and the system contained beds planted with *Typha Australis* and the other systems was controlled system, observed without using plants. The filtration system used materials with high sorption sites, like charcoal, which encouraged the growth of pathogens.

Table 3.5: Design elements and size of each Constructed Wetland system (CWs)

No. of CW System	Height (m)	Diameter (m)	Retention time (HTR)
1	0.6	0.2	48 hrs.
2	0.6	0.2	48 hrs.
3	0.6	0.2	48 hrs.
4(Control System)	0.6	0.2	48 hrs.

Table 3.6: Name of plants utilized in Constructed Wetland system (CWs)

Plants used in Wetland Systems	
Common Names	Scientific Names
Cattail/bulrush	Typha Australis
Water Hyacinth	Eichhornia crassipes

Constructed wetlands (CWs) are engineered systems designed to utilize natural processes involving wetland vegetation, soils, and associated microbial communities to treat wastewater. They are increasingly recognized as a sustainable and cost-effective alternative to conventional wastewater treatment methods. CWs can effectively remove a variety of pollutants, including nutrients, organic matter, and emerging contaminants, by mimicking the natural filtration and absorption processes found in natural wetlands. Their design can vary significantly based on the specific treatment goals, local climate, and available space (Zhao, et al., 2022). In the context of wastewater treatment, three distinct types of constructed wetlands have been developed to address varying concentrations of synthetic wastewater and to operate under different time intervals.

The system 1 of CW system incorporated the plant *Typha australis*, commonly known as common reed. This wetland was designed with the same 4 material layers that supported the growth of this emergent plant. *Typha australis* is known for its robust growth and ability to uptake nutrients and contaminants from the water, making it an effective biological component in the treatment process. The presence of vegetation not only aids in pollutant removal but also enhances habitat for various microorganisms that contribute to the degradation of organic matter.

Figure 3.1(a): Plant Typha Australis

Figure 3.1(b): First CW system with Typha Australis

The system 2 of constructed wetland system was only characterized by a four layered substrate consisting of gravel, charcoal, fine gravels, and sand. No plants were added in this system. The layers serve multiple functions: the gravel provides structural support and facilitates water flow, while the charcoal can adsorb organic pollutants and enhance microbial activity. The fine gravels and sand layers help in filtering out smaller particles, improving the overall treatment efficiency.

Figure 3.2: Second Wetland system with beds

The system 3 of constructed wetland utilized Water Hyacinth (*Eichhornia crassipes*). Unlike the previous types, this wetland does not have a structured four layered substrates. Instead, the Water Hyacinth floats on the surface of the water, where it can absorb nutrients and contaminants directly from the water column. This type of wetland can particularly be effective in treating wastewater with high nutrient loads, as the rapid growth of Water Hyacinth can significantly reduce nutrient concentrations through uptake and biomass production.

Figure 3.3: Floating Plant Wetland with Water Hyacinth

These constructed wetland designs demonstrate the versatility of CWs in treating wastewater under varying conditions and highlight the importance of selecting appropriate plant species and substrate materials to optimize treatment performance.

Figure 3.4: All three constructed wetlands with pure control wetland

3.4 Experimental framework of constructed wetlands (CWs)

A total of three batches were run, and all batches were compared with control samples to assess the efficiency of each constructed wetland. For the first batch, 6 liters of synthetic wastewater were added into each constructed wetland and tested over a period of 15 days. In the second batch, 7 liters of synthetic wastewater were introduced into each constructed wetland. For the third batch, 8 liters of synthetic wastewater were added to each constructed wetland. Different volume of wastewater was used to check the efficiency of each wetland system at different concentrations and different time duration. Sampling for all three batches was conducted every 48 hours to measure the concentrations of pH, Total Dissolved Solids (TDS), Biochemical Oxygen Demand (BOD), Dissolved Oxygen (DO), and Chemical Oxygen Demand (COD). An empty container was also filled with the same amount to serve as a control, and this control sample was similarly sampled every 48 hours throughout the 15-day period. Through these systematic approaches across the three batches, the study aims to evaluate the performance of different constructed wetland designs in treating pharmaceutical wastewater effectively.

3.5 Sampling of wastewater

For the sampling of wastewater plastic bottles were utilized. 500ml of wastewater samples were taken drop by drop from three systems including the control system as well. Seven samples (seven bottles) from each wetland system were taken. Samples collection was done every 48 hours from inlets and outlets of every wetland in separate bottles.

Figure 3.5: Drop by drop wastewater grab sampling from all three wetlands

The bottles were carefully sealed and marked according to the type of the constructed wetland and batch number including the samples from the control system as well. This process ensures that the samples were collected and stored systematically, allowing for accurate analysis and comparison of the wastewater quality across different constructed wetlands and the control system.

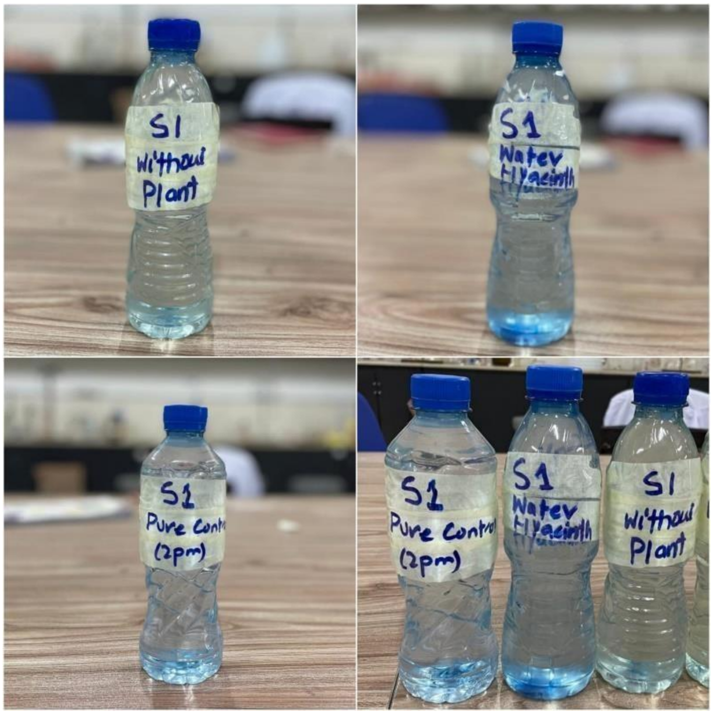

Figure 3.6: Labelled wastewater samples taken from all wetlands and control system

3.6 Methods of sample analysis

Chemical estimation of collected wastewater samples was done by using following parameters: pH, Chemical Oxygen Demand (COD), 5 days Biochemical Oxygen Demand (BOD), Total Dissolved Solids (TDS), and Dissolved Oxygen (DO). The sampling protocols for these parameters are given below:

3.6.1 Estimation of pH

The pH of the samples was determined by employing a pH meter. To guarantee precise results and prevent any potential cross-contamination, the pH meter's probe was thoroughly cleansed with distilled water prior to each measurement. In order to prevent any interference from previous samples, the beaker that was utilized to contain the water sample was also cleansed with distilled water.

Reagents

1. Distilled water
2. Water sample

Procedure

Before analyzing our samples, the pH meter was calibrated using standards. A graduated cylinder was utilized to measure a 50 ml water sample, which was then transferred to a beaker. Carefully pouring the water sample into the cleansed beaker, the pH meter's probe was submerged in the sample for a few minutes. After a sufficient amount of time had passed for stabilization, the pH reading was recorded.

3.6.2 Total dissolved solids (TDS)

The TDS meter was employed to measure the total dissolved solids (TDS) in the water samples, adhering to an identical protocol. The TDS meter's probe was submerged in the sample for a few minutes after the water sample was meticulously transferred into the rinsed beaker. The TDS readings were recorded after a substantial amount of time had passed to allow for stabilization.

3.6.3 Chemical Oxygen Demand (COD)

Materials

- COD Vials
- Sample: Wastewater sample to be tested.
- Potassium Hydroxide Pellets
- Magnetic Stirrer:
- Oven: Preheated to 150°C for refluxing the samples.
- Spectrophotometer: For measuring the absorbance of the digested sample.
- Cooling Bath: To cool the vials after digestion.

The method for assessing Chemical Oxygen Demand (COD), which quantifies the molecular oxygen consumed in the oxidation of organic compounds inside a sample, was executed under controlled conditions at elevated temperatures for a specified duration. The BOD5 value often accounts for 25-50% of the COD. The procedure for the analysis was as follows:

Procedure

A COD vial with the standard digestion solution was initially obtained. Subsequently, 50 ml of the material was introduced into the container. The tube was securely sealed, and the contents were fully combined. The oven was preheated to 150°C, and the tubes were positioned inside to reflux for two hours. Subsequent to refluxing, the tubes were permitted to cool before being positioned in the spectrophotometer cell holder, with the lid secured to finalize the operation.

3.6.4 Biological Oxygen Demand (BOD)

Materials

- BOD Bottles: (500 ml glass)
- BOD Nutrient Buffer Pillow
- Potassium Bromide (KBr)
- Incubator: Set at 20°C for the five-day incubation period.
- Magnetic Stirrer: To ensure thorough mixing of the sample
- Thermometer: To monitor the temperature of the sample.
- Gloves and Safety Goggles: For personal protection during handling and analysis.

The assessment of biologically oxidized organic matter in wastewater samples was performed via an indirect empirical analysis utilizing the Biochemical Oxygen Demand (BOD) test, a standard method for evaluating waste loading and wastewater treatment efficacy. The procedure for the analysis is given below:

Procedure

A 300 ml sample of wastewater was collected. A BOD nutrient buffer pillow was subsequently incorporated into the sample, along with potassium hydroxide pellets and a magnetic stir bar to facilitate continual agitation. The sample vial was thereafter positioned in an incubator maintained at 20°C for five days. Upon confirming that the bottles were securely sealed, and the apparatus was operating correctly, the measurements were evaluated following the five-day incubation period.

3.6.5 Dissolved oxygen (DO)

Materials Required

- Dissolved oxygen meter (calibrated)
- Wastewater sample
- Clean sampling container (Glass)
- Stirring rod or magnetic stirrer
- Thermometer
- Gloves and safety goggles

Procedure

The process for measuring dissolved oxygen (DO) in wastewater using a DO meter involved multiple steps. The DO meter was calibrated using standard solutions with known dissolved oxygen concentrations. A standard wastewater sample was obtained in a sterile container, and its temperature was recorded. The DO meter probe was thereafter immersed in the wastewater, achieving full submersion without touching the bottom, while the sample was gently agitated to promote equal oxygen distribution. After the stabilization of the reading, the concentration of dissolved oxygen was quantified in milligrams per liter (mg/L). The measurement was repeated to ensure accuracy, and the probe was then rinsed with distilled water. The recorded dissolved oxygen levels were analyzed for the efficacy of wastewater treatment, complying with all safety protocols during the process (He, et al., 2018).

3.7 High-Performance Liquid Chromatography (HPLC)

After the experiment, the samples were further analyzed for HPLC to ascertain the levels of pharmaceuticals in wastewater to see which constructed wetland is more efficient and effective in removing the pharmaceuticals. The medications Ciprofloxacin, Cefixime, Ibuprofen, and Paracetamol were subjected to analysis via High-Performance Liquid Chromatography (HPLC). HPLC is a widely utilized analytical technique for precisely quantifying antibiotics in various matrices, including plant tissues and wastewater. The procedure for assessing the absorption of antibiotics by plants and estimating the amount derived from wastewater encompassed the following steps (Özengin & Elmaci, 2016).

The HPLC protocol utilized for analyzing the levels of pharmaceuticals in wastewater involved a mobile phase composed of phosphate buffer at pH 6.8 and methanol in a 60:40 ratio. The chromatographic conditions were set to operate in liquid chromatography mode, using a C18 column measuring 25 cm × 4.6 mm with a particle size of 5 micrometers. The flow rate was maintained at 1 ml per minute, and detection was performed at 254 nm using UV spectroscopy. An injection volume of 20 microliters was employed for the samples. To calculate the percentage reduction of pharmaceuticals, the formula used was the peak area of the samples divided by the peak area of the control, multiplied by 100. The formula is given by:

Reduction (Percentage %) = Peak area of the samples ÷ Peak area of the control × 100 (Muhammad, et al., 2017)

3.7.1 Removal Efficiency Calculation

The removal efficiencies of antibiotics from the wastewater were calculated using the formula:

Removal Efficiency (%) = $(C\text{in} - C\text{out}/\ C\text{in}) \times 100$

Where:

- Cin = concentration of antibiotics in the influent (wastewater before treatment)
- Cout = concentration of antibiotics in the effluent (wastewater after treatment) (Hijosa-Valsero, et al., 2016)

3.8 Data analysis

Descriptive statistics were applied to the data (mean and standard deviation). The Multifactor analysis of variance (ANOVA) Will be applied to the removal efficiencies. The least significant difference (LSD) test for differences between means was used in multiple comparisons when a significant difference between treatments was found during the ANOVA procedure.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Timeframe 1 (Batch 1) – Days 1-15

Tables 4.1, 4.2, and 4.3 provide an extensive overview of the treatment efficiencies of three distinct constructed wetland systems, each utilizing different plant species: BEDS, Water Hyacinth, and Typha Australis. The tables below showed summary of the findings, specifically emphasizing the decrease in Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD), as well as the noted shifts in pH, DO, and Total Dissolved Solids (TDS).

4.1.1 Overall performance of system 1 planted with *Typha Australis*

Table 4.1: Overall Performance of the System 1(TYPHA AUSTRALIS) for Batch 1(1-15days)

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with TYPA AUSTRALIS)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	257.1	88.3±24.75	65.61
2	BOD	mg/l	7	218.3	71.7±15.57	67.1
3	DO	mg/l	7	8.9	14.1±4.42	
4	TDSS	mg/l	7	916.25	435±165.3	40
5	PH	mg/l	7	4.72	6.9±0.362	

In the system 1 planted with Typha Australis COD reduced from 257.1 mg/l to 88.3 mg/l (65.61% reduction). BOD reduced from 218.3 mg/l to 71.3 mg/l (67.1% reduction). The shifts in the levels of TDS, DO, and pH were far more pronounced than those observed in the next two systems. The pH levels were increased from more acidic (4.72) to basic (6.90) readings. The TDS levels decreased from 916.25 mg/L to 435 mg/L. Additionally, the levels of DO were considerably increased from 8.9 mg/L to 14.1 mg/L. The graph for each parameters pH, TDS, BOD, COD, and DO are represented here after:

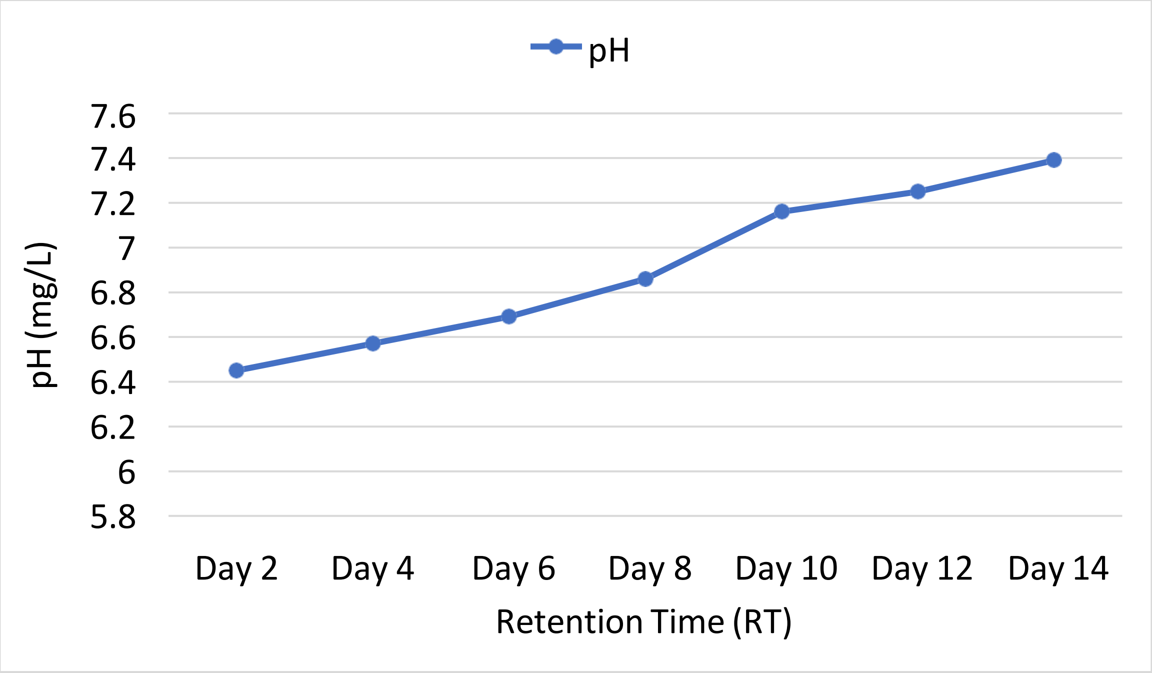

Figure 4.1(a): Day wise change in pH for system 1 (Typha Australis) of Batch 1 (1-15days)

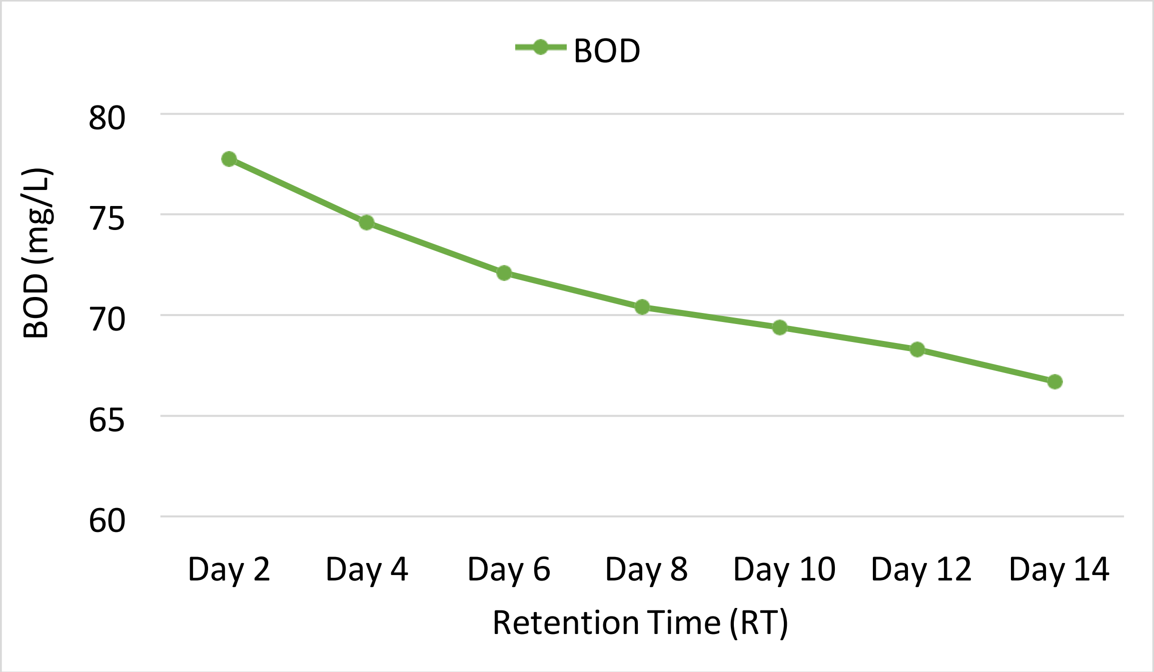

Figure 4.1(b): Day wise change in BOD for system 1 (Typha Australis) of Batch 1 (1-15days)

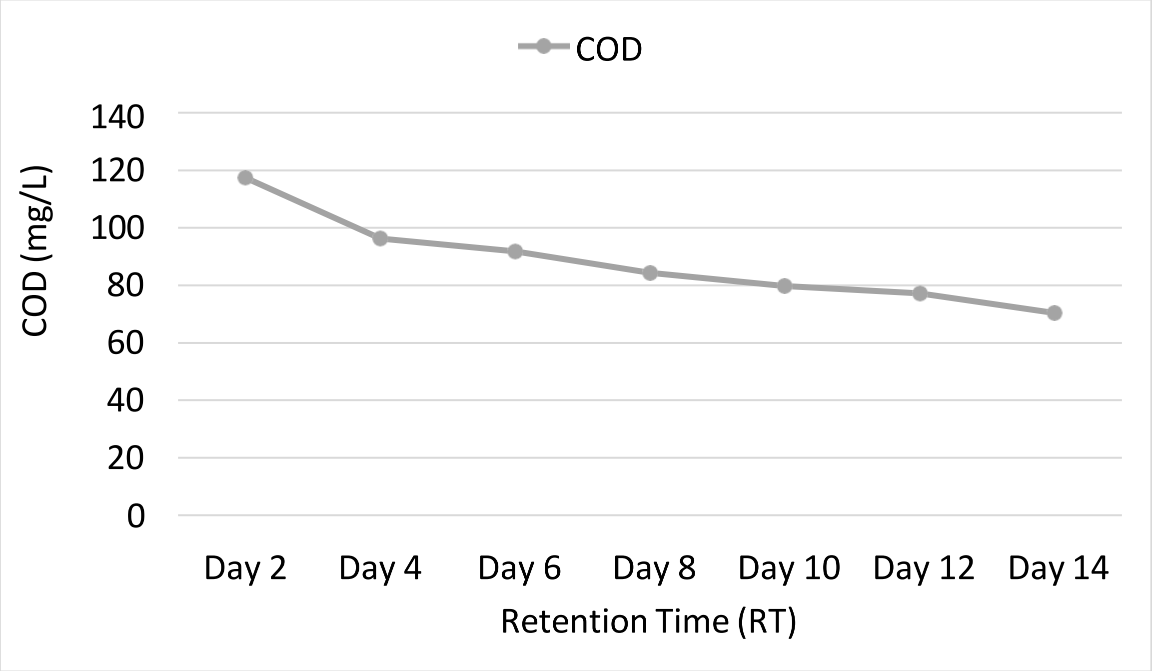

Figure 4.1(c): Day wise change in COD for system 1 (Typha Australis) of Batch 1 (1-15days)

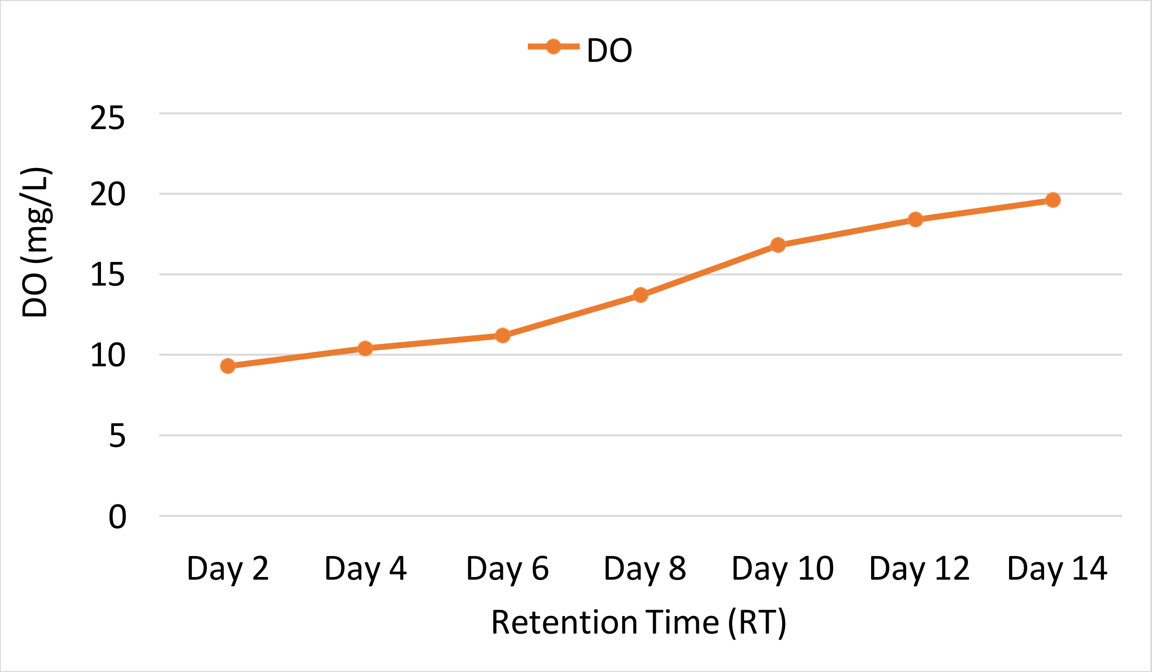

Figure 4.1(d): Day wise change in DO for system 1 (Typha Australis) of Batch 1 (1-15days)

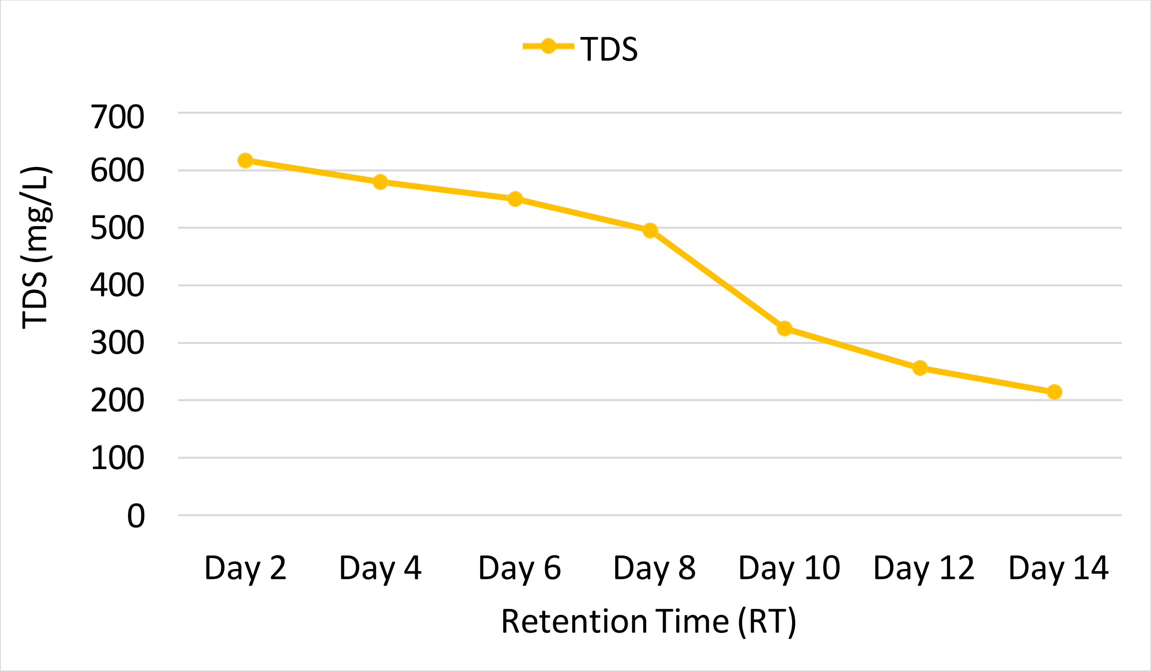

Figure 4.1(e): Day wise change in TDS for system 1 (Typha Australis) of Batch 1 (1-15days)

4.1.2 Overall performance of system 2 planted with beds

In the system 2 with beds, COD reduced from 257.1 mg/l to 107.3 mg/l (58.2% reduction) whereas BOD reduced from 218.3 mg/l to 94.6 mg/l (56.6% reduction). The pH levels were increased from more acidic (4.72) to basic (6.73) readings. The TDS levels decreased from 916.25 mg/L to 536 mg/L. The levels of DO were considerably increased from 8.9 mg/L to 12.6 mg/L. The graph for each parameters pH, TDS, BOD, COD, and DO of system 2 are represented here after:

Table 4.2: Overall Performance of the System 2 for Batch 1

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with BEDS)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	257.1	107.3 ± 17.8	58.2
2	BOD	mg/l	7	218.3	94.6±14.95	56.6
3	DO	mg/l	7	8.9	12.8±3.61	
4	TDSS	mg/l	7	916.25	536±178.6	42.7
5	PH	mg/l	7	4.72	6.73±0.348	

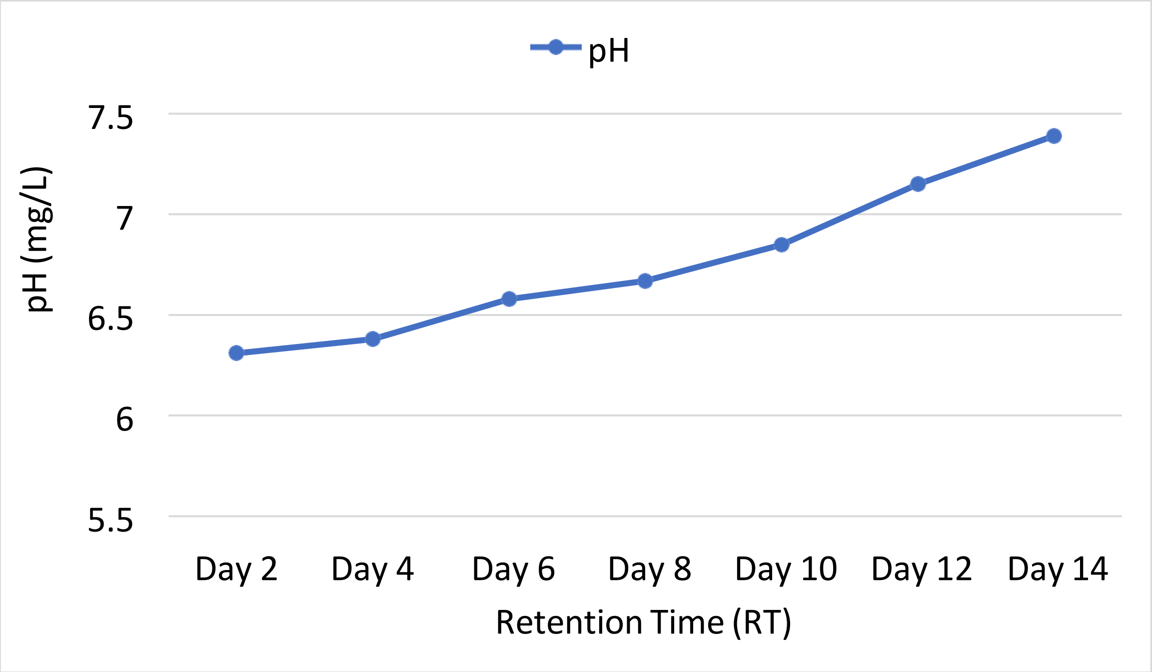

Figure 4.2(a): Day wise change in pH for system 2 (Beds) of Batch 1 (1-15days)

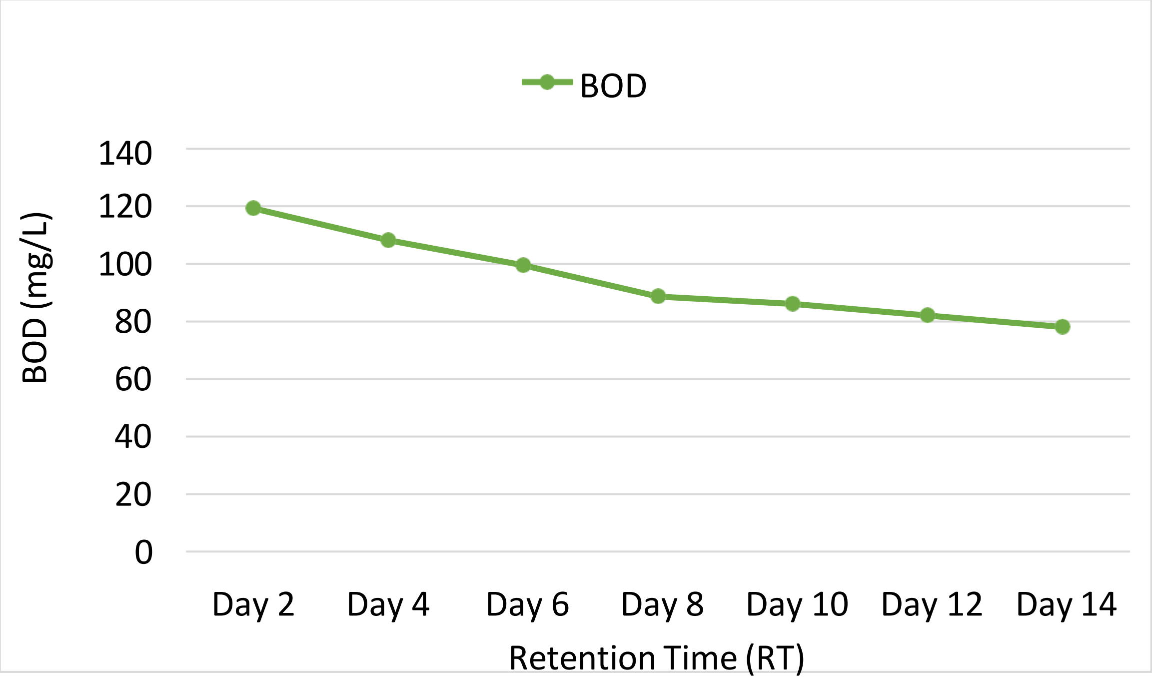

Figure 4.2(b): Day wise change in BOD for system 2 (Beds) of Batch 1 (1-15days)

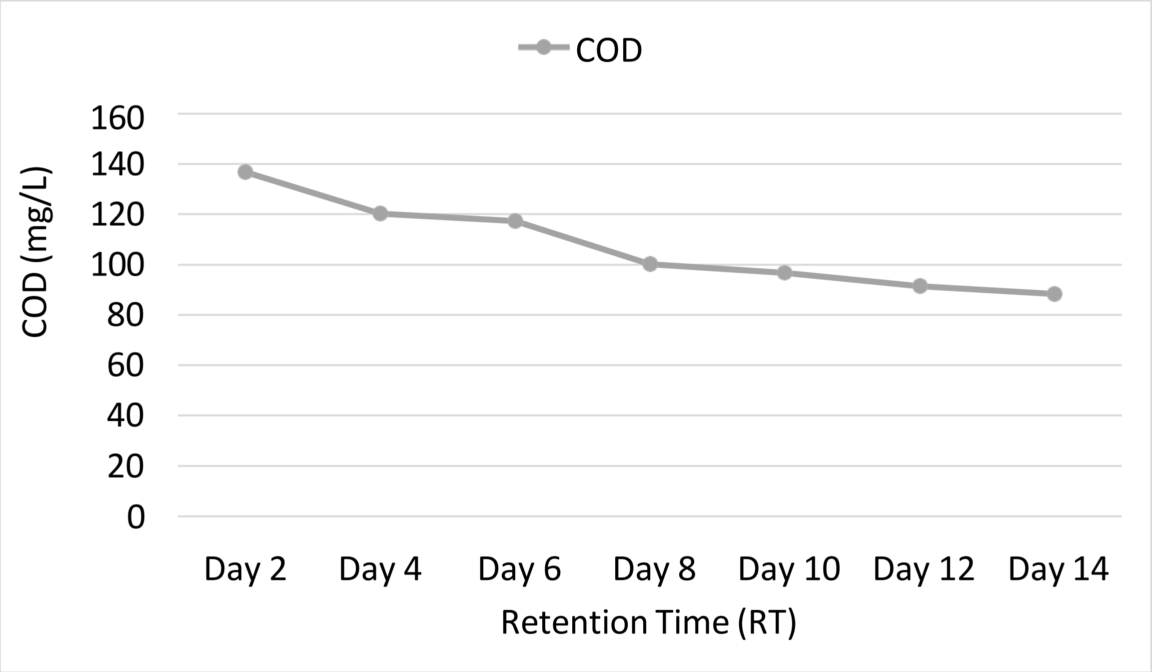

Figure 4.2(c): Day wise change in COD for system 2 (Beds) of Batch 1 (1-15days)

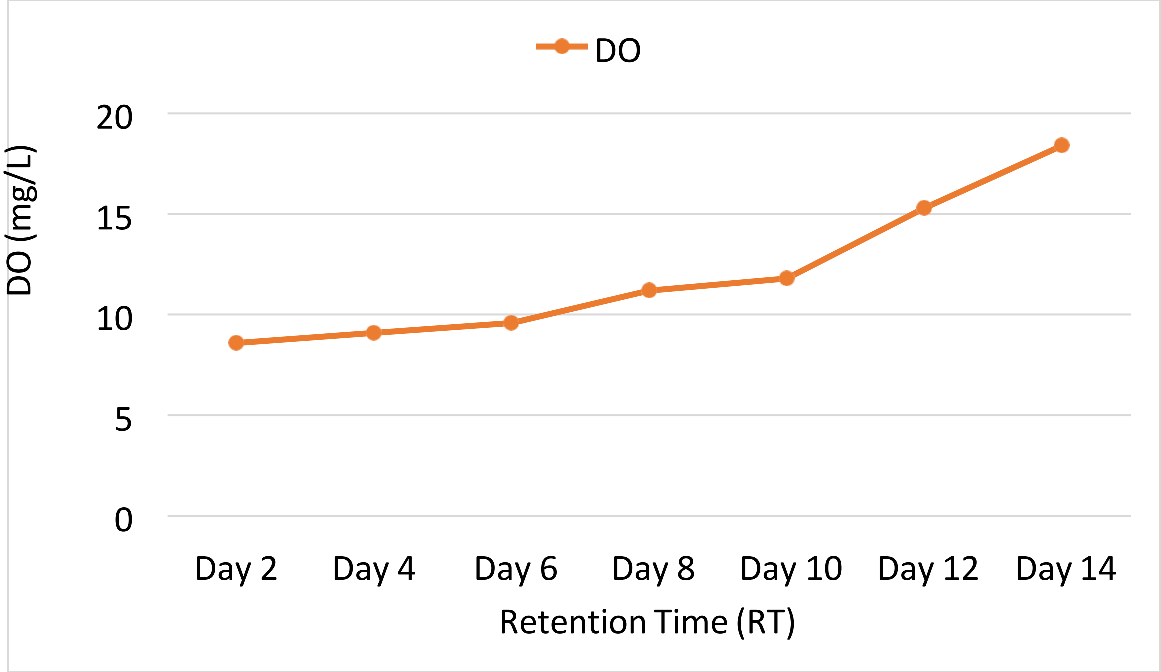

Figure 4.2(d): Day wise change in DO for system 2 (Beds) of Batch 1 (1-15days)

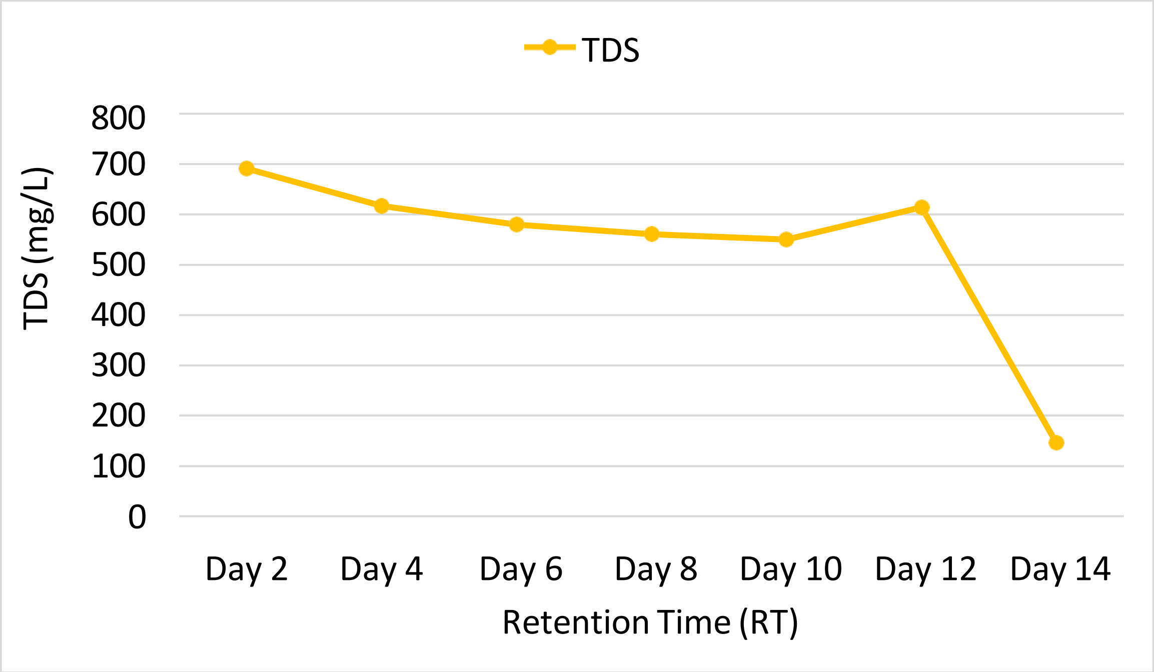

Figure 4.2(e): Day wise change in TDS for system 2 (Beds) of Batch 1 (1-15days)

4.1.3 Overall performance of system 3 planted with *Water Hyacinth*

In system 3 planted with *Water Hyacinth*, the COD reduced from 257.1 mg/l to 138.9 mg/l (46.2% reduction) whereas BOD reduced from 218.3 mg/l to 100.7 mg/l (53.87% reduction). The shifts in the levels of TDS, DO, and pH were less pronounced than those observed in the initial two systems. The pH levels were increased from 4.72 mg/L to 6.41 mg/L. The TDS levels decreased from 916.25 mg/L to 464 mg/L. The levels of DO were considerably increased from 8.9 mg/L to 10.7 mg/L. The graph for each parameters pH, TDS, BOD, COD, and DO of system 3 are represented here after:

Table 4.3: Overall Performance of the System 3(WATER HYACINTH) for Batch 1

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with WATER HYACINTH)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	257.1	138.9 ± 33.15	46.2
2	BOD	mg/l	7	218.3	100.7±25.3	53.87
3	DO	mg/l	7	8.9	10.7±2.77	
4	TDSS	mg/l	7	916.25	464±167.2	49.1
5	PH	mg/l	7	4.72	6.41±0.331	

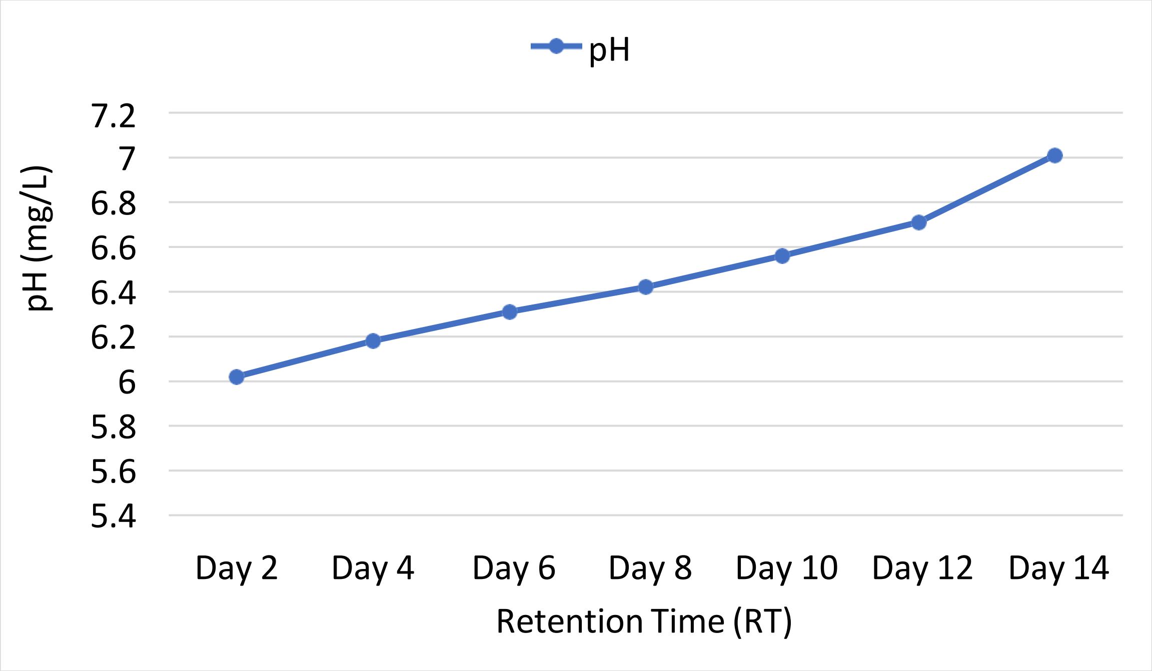

Figure 4.3(a): Day wise change in pH for system 3 (Water Hyacinth) of Batch 1 (1-15days)

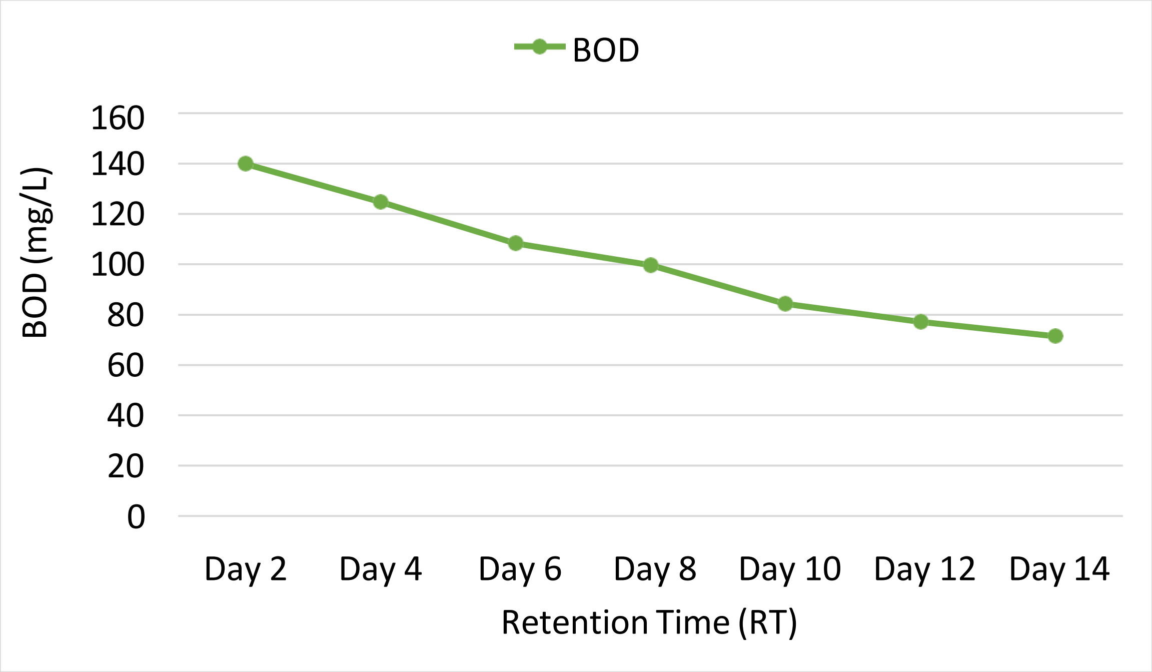

Figure 4.3(b): Day wise change in BOD for system 3 (Water Hyacinth) of Batch 1 (1-15days)

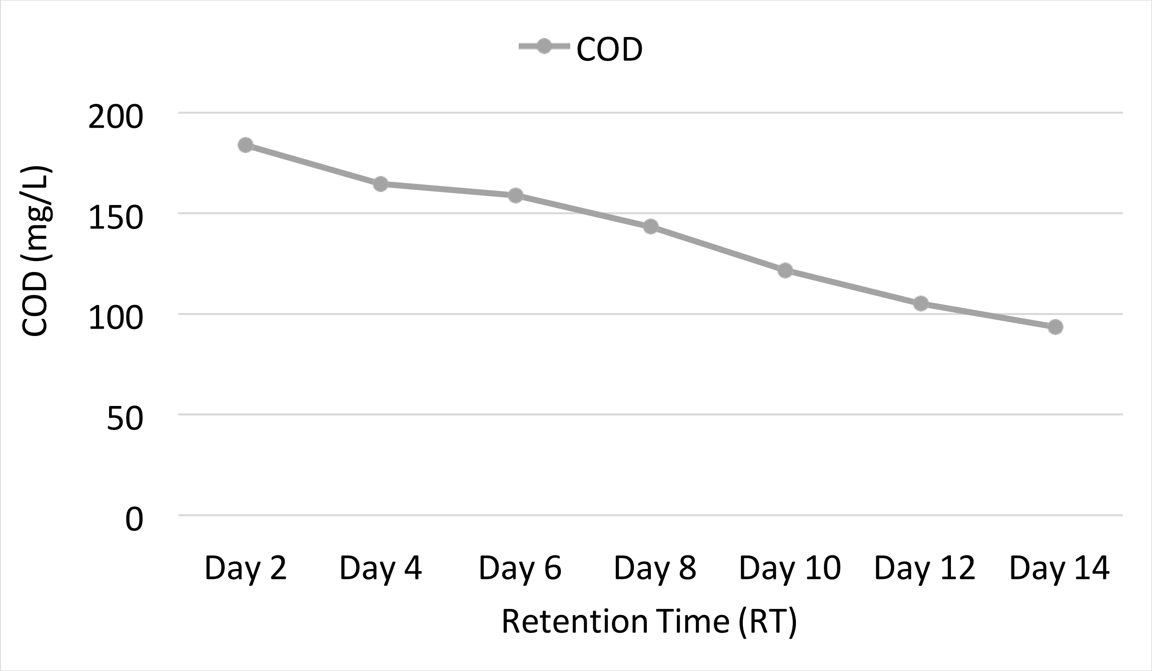

Figure 4.3(c): Day wise change in COD for system 3 (Water Hyacinth) of Batch 1 (1-15days)

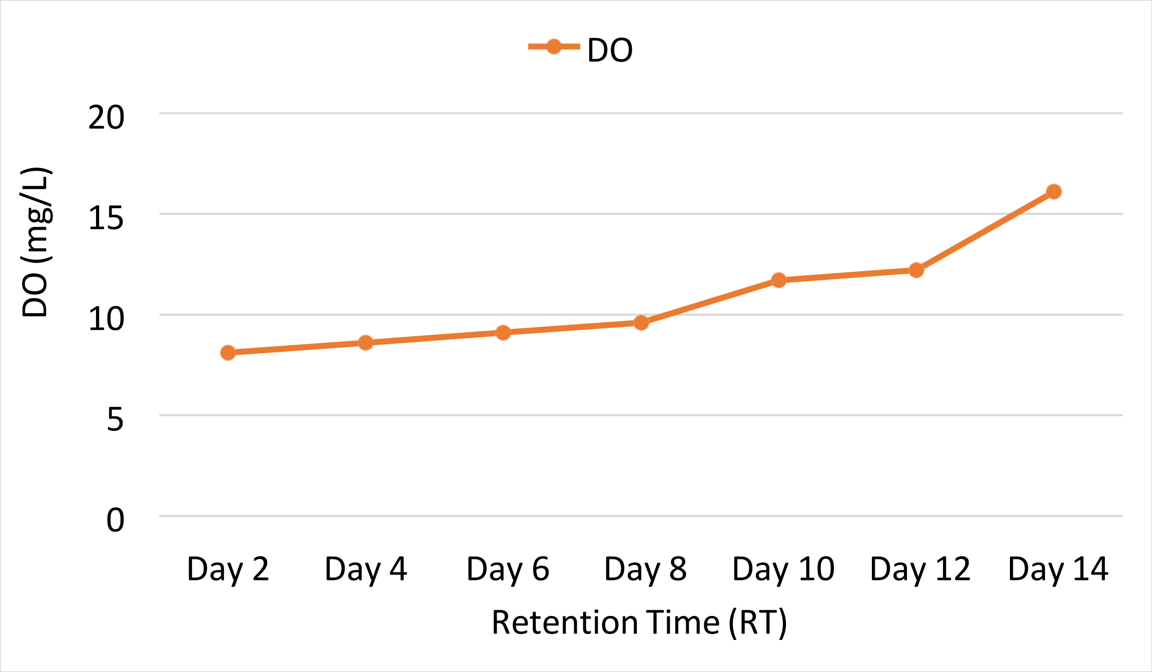

Figure 4.3(d): Day wise change in DO for system 3 (Water Hyacinth) of Batch 1 (1-15days)

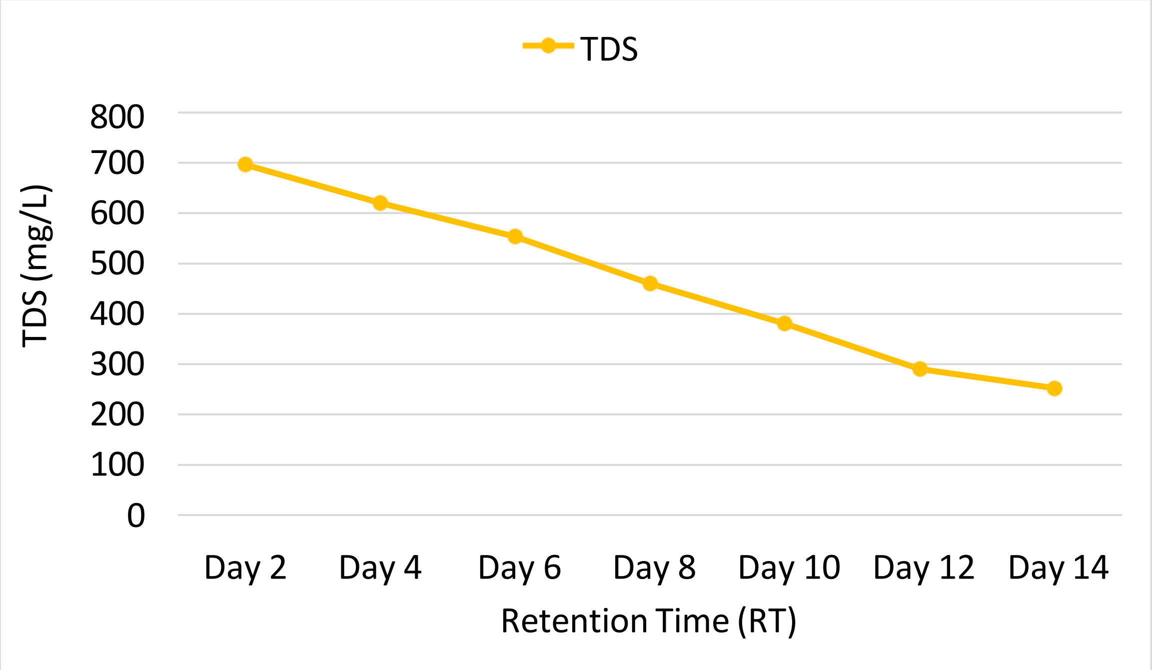

Figure 4.3(e): Day wise change in TDS for system 3 (Water Hyacinth) of Batch 1 (1-15days)

The pH levels elevated in all systems, signifying a transition to more alkaline (basic) environments. This augmentation is due to the biological activities that occurred in the wetlands. The decomposition of organic substances by microbial activity produced alkaline byproducts that elevated the pH. Moreover, the existence of specific plants augmented this increase by sequestering acidic chemicals and emitting oxygen, thereby increasing the pH. Total Dissolved Solids (TDS) exhibited reduction over time among the systems. The reduction of Total Dissolved Solids (TDS) over time can be linked to several basic mechanisms. Sedimentation facilitated the deposition of denser particles from the water, but plant absorption diminished the dissolved solids as plants incorporated nutrients and minerals. Moreover, microbial breakdown degraded the organic materials, transforming some dissolved particles into biomass. Collectively, these activities provided the efficient decrease of TDS, hence improving the overall water quality within the system.

The increase in Dissolved Oxygen (DO) levels in constructed wetland systems could be attributed to several factors. Firstly, the photosynthetic activity of aquatic plants, such as Typha Australis and Water Hyacinth, contributed significantly to oxygen production during daylight hours. Additionally, the turbulent flow of water through the wetland enhanced aeration, allowing for greater oxygen exchange with the atmosphere.

4.2 Timeframe 2 (Batch 2) – Days 15-30

Tables 4.4, 4.5, and 4.6 provide an extensive overview of the treatment efficacy of three distinct constructed wetland systems, each utilizing different plant species: BEDS, *Water Hyacinth, and Typha Australis.* The tables below showed summary of the findings, specifically emphasizing the decrease in Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD), as well as the noted shifts in pH, DO, and Total Dissolved Solids (TDS).

4.2.1 Overall performance of system 1 planted with *Typha Australis*

In the system 1 planted with *Typha Australis* COD reduced from 283.1 mg/l to 104.3 mg/l (63.2% reduction). BOD reduced from 236.3 mg/l to 81.6 mg/l (64.9% reduction). The variations in TDS, DO, and pH levels were somewhat less significant than those noted in the initial batch. The pH levels were slightly increased from acidic (5.18) to basic (6.60) readings. The TDS levels greatly decreased from 986.2 mg/L to 494.3 mg/L. Additionally, the levels of DO were considerably increased from 9.6 mg/L to 12.5 mg/L. The table below shows the mean± SD values for each parameters pH, TDS, BOD, COD, and DO:

Table 4.4: Overall Performance of the System 1 for Batch 2

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with TYPA AUSTRALIS)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	283.1	104.3±18.6	63.2
2	BOD	mg/l	7	236.3	81.6±16.2	64.9
3	DO	mg/l	7	9.6	12.5±3.57	
4	TDSS	mg/l	7	986.2	494.3±`18.4	50.4
5	PH	mg/l	7	5.18	6.60±0.341	

4.2.2 Overall performance of system 2 planted with Beds

In the system 2 with beds, COD reduced from 283.1 mg/l to 123.3 mg/l (55.6% reduction) whereas BOD reduced from 236.3 mg/l to 112.6 mg/l (52.10% reduction). The pH levels were slightly increased from acidic (5.19) to basic (6.41) readings. The TDS levels were greatly decreased from 986.2 mg/L to 605 mg/L. The levels of DO were slightly increased from 9.6 mg/L to 11.6 mg/L. The variations in TDS, DO, and pH levels were somewhat less significant than those noted in the initial batch. The table below shows the mean± SD values for each parameters pH, TDS, BOD, COD, and DO:

Table 4.5: Overall Performance of the System 2 for Batch 2

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with BEDS)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	283.1	123.3±22.8	55.6
2	BOD	mg/l	7	236.3	112.6±26.7	52.1
3	DO	mg/l	7	9.6	11.6±3.1	
4	TDSS	mg/l	7	986.2	605±186,4	38.5
5	PH	mg/l	7	5.19	6.4±0.331	

4.2.3 Overall performance of system 3 planted with *Water Hyacinth*

In system 3 planted with *Water Hyacinth*, the COD reduced from 283.1 mg/l to 156.9 mg/l (44.5% reduction) whereas BOD reduced from 236.3 mg/l to 115.4 mg/l (51.9% reduction). The variations in TDS, DO, and pH levels were somewhat less significant than those noted in the initial batch. The pH levels were slightly increased from 5.19 mg/L to 6.23 mg/L. The TDS levels were greatly decreased from 986.2 mg/L to 518.3 mg/L. The levels of DO were slightly increased from 9.6 mg/L to 10.9 mg/L. The table below shows the mean± SD values for each parameters pH, TDS, BOD, COD, and DO:

Table 4.6: Overall Performance of the System 3 for Batch 2

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with WATER HYACINTH)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	283.1	156.9±36.8	44.5
2	BOD	mg/l	7	236.3	115.4±28.4	51.9
3	DO	mg/l	7	9.6	10.9±2.81	
4	TDSS	mg/l	7	986.2	518.3±156.2	47.6
5	PH	mg/l	7	5.18	6.23±0.273	

4.3 Timeframe 3 (Batch 3) – Days 30-45

Tables 4.7, 4.8, and 4.9 provide an extensive overview of the treatment efficacy of three distinct constructed wetland systems, each utilizing different plant species: BEDS, *Water Hyacinth, and Typha Australis* for batch three. The tables below showed summary of the results, specifically the decrease in Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD), as well as the noted shifts in pH, DO, and Total Dissolved Solids (TDS).

4.3.1 Overall performance of system 1 planted with *Typha Australis*

In the system 1 planted with *Typha Australis* COD reduced from 283.1 mg/l to 104.3 mg/l (63.2% reduction). BOD reduced from 236.3 mg/l to 81.6 mg/l (64.9% reduction).

Table 4.7: Overall Performance of the System 1 for Batch 3

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with TYPA AUSTRALIS)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	296.4	116.9±22.7	60.2
2	BOD	mg/l	7	248	90.3±13.96	65.7
3	DO	mg/l	7	9.9	11.3±3.13	
4	TDSS	mg/l	7	1004	518.3±167.9	48.9
5	PH	mg/l	7	5.41	6.35±0.279	

The variations in TDS, DO, and pH levels were somewhat less significant than those noted in the initial batch. The pH levels were slightly increased from acidic (5.18) to basic (6.60) readings. The TDS levels greatly decreased from 986.2 mg/L to 494.3 mg/L. Additionally, the levels of DO were considerably increased from 9.6 mg/L to 12.5 mg/L. The table below shows the mean± SD values for each parameters pH, TDS, BOD, COD, and DO:

4.3.2 Overall performance of system 2 planted with beds

In the system 2 with beds, COD reduced from 296.4 mg/l to 172.3 mg/l (41.5% reduction) whereas BOD reduced from 248 mg/l to 122.6 mg/l (50.2% reduction). The pH levels were slightly increased from acidic (5.41) to basic (6.28) readings. The TDS levels were greatly decreased from 1004 mg/L to 640.3 mg/L. The levels of DO were slightly increased from 9.9 mg/L to 10.9 mg/L. The variations in TDS, DO, and pH levels were somewhat less significant than those noted in the initial batch. The table below shows the mean± SD values for each parameters pH, TDS, BOD, COD, and DO:

Table 4.8: Overall Performance of the System 2 for Batch 3

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with BEDS)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	296.4	172.3± 42.4	41.5
2	BOD	mg/l	7	248	122.6±28.7	50.2
3	DO	mg/l	7	9.9	10.9±2.78	
4	TDSS	mg/l	7	1004	640.3±124.7	36.4
5	PH	mg/l	7	5.41	6.28±0.275	

4.3.3 Overall performance of system 3 planted with *Water Hyacinth*

In system 3 planted with *Water Hyacinth*, the COD reduced from 296.4 mg/l to 156.9 mg/l (44.5% reduction) whereas BOD reduced from 248 mg/l to 115.4 mg/l (51.4% reduction). The variations in TDS, DO, and pH levels were somewhat less significant than those noted in the initial batches. The pH levels were slightly increased from 5.41 mg/L to 6.15 mg/L. The TDS levels were greatly decreased from 1004 mg/L to 559.6 mg/L. The levels of DO were slightly increased from 9.9 mg/L to 9.41 mg/L. The table below shows the mean± SD values for each parameters pH, TDS, BOD, COD, and DO:

Table 4.9: Overall Performance of the System 3 for Batch 3

	Water Quality Variables (Mean # Standard Deviation) for the Constructed Wetland (planted with WATER HYACINTH)					
S. No	Variables	Unit	N	Inlet	Outlet	Reduction
1	COD	mg/l	7	296.4	156.9 ± 38,3	44.5
2	BOD	mg/l	7	248	115.4±25.6	51.4
3	DO	mg/l	7	9.9	9.41±2.62	
4	TDSS	mg/l	7	1004	559.6±198.7	44.8
5	PH	mg/l	7	5.41	6.15±0.261	

The efficacy of various constructed wetland systems for synthetic wastewater treatment underscores the vital necessity of complying with the National Environmental Quality Standards (NEQS) for wastewater. The research illustrated substantial decreases in pollutant concentrations through the systematic monitoring of critical parameters, including Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Dissolved Solids (TDS), pH, and Dissolved Oxygen (DO). This highlighted the efficacy of systems incorporating species such as Water Hyacinth and Typha Australis in achieving or surpassing NEQS standards. This alignment highlighted the capacity of synthetic wetlands to enhance water quality and safeguard wetland ecosystems, while also highlighting their significance in advancing sustainable wastewater management methods. The results supported the extensive use of these sustainable systems, offering critical information for policymakers to improve environmental health and adherence to national wastewater treatment standards in Pakistan.

4.4 Detection of concentration of pharmaceuticals present in wastewater using HPLC

The High-Performance Liquid Chromatography (HPLC) analysis was performed to determine the rate of removal of pharmaceuticals in synthetic wastewater systems and to assess the efficacy of each created wetland system across three separate batches. Seven samples were obtained from each system in each batch, with samples 3, 5, and 7 from each system analyzed via HPLC to ascertain the concentration of the drugs present. The generated graphs demonstrate the percentage of pharmaceuticals, specifically antibiotics, in the wastewater for each batch across all three systems planted with Typha Australis, Water Hyacinth, and Beds. The initial batch exhibited the highest clearance rates, signifying higher treatment efficacy. The Typha Australis exhibited the highest efficacy in medicine removal across all three batches, preceded by the Beds and Water Hyacinth system. The removal efficiency was consistently ordered over the batches as follows: Batch 1 > Batch 2 > Batch 3. For the plant species and constructed wetland systems, the order of significance was Typha Australis > Water Hyacinth > Beds. This underscored the efficacy of Typha Australis and Water Hyacinth in improving the treatment of synthetic wastewater contaminated with pharmaceuticals. The graphs for each batch illustrating the percentage of medication across the three distinctive systems are shown below:

4.4.1 Results of Batch 1(1-15 days)

In Batch 1 of the study, medication removal rates by each designed wetland system were determined based on the percentage of medications present in the wastewater. The Typha Australis system exhibited efficient removal, attaining 42% removal of medicines from Sample 3, 43.5% from Sample 5, and 43% from Sample 7. The Water Hyacinth system demonstrated removal rates, attaining 35.6% for Sample 3, 37.0% for Sample 5, and 36.3% for Sample 7. Conversely, the Beds system exhibited decreasing removal efficiency, achieving 32.5% medication removal from Sample 3, 31.3% from Sample 5, and 32.6% from Sample 7. The results underscored the varied efficacy of each system, with Typha Australis demonstrating best removal rates in Batch 1. The other two systems also showed considerable efficiency in eliminating medicines from the effluent.

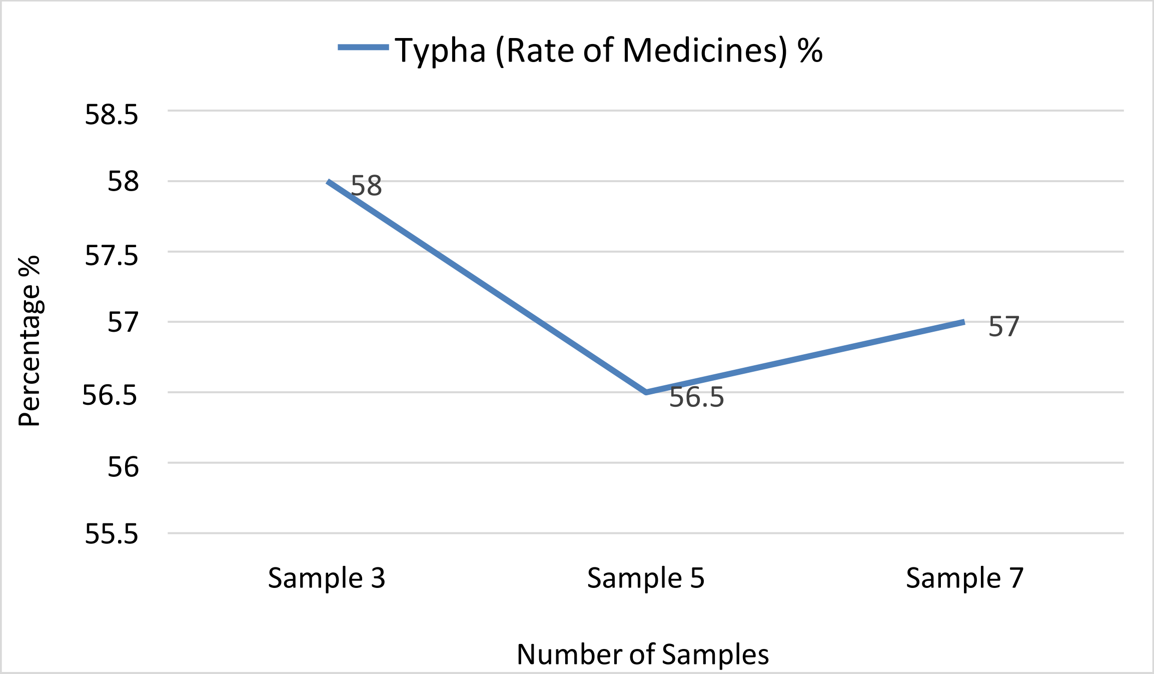

Figure 4.4 (a): concentration of pharmaceutical compound present in wastewaters (%) in system 1 (Typha Australis) of Batch 1(1-15days) using HPLC

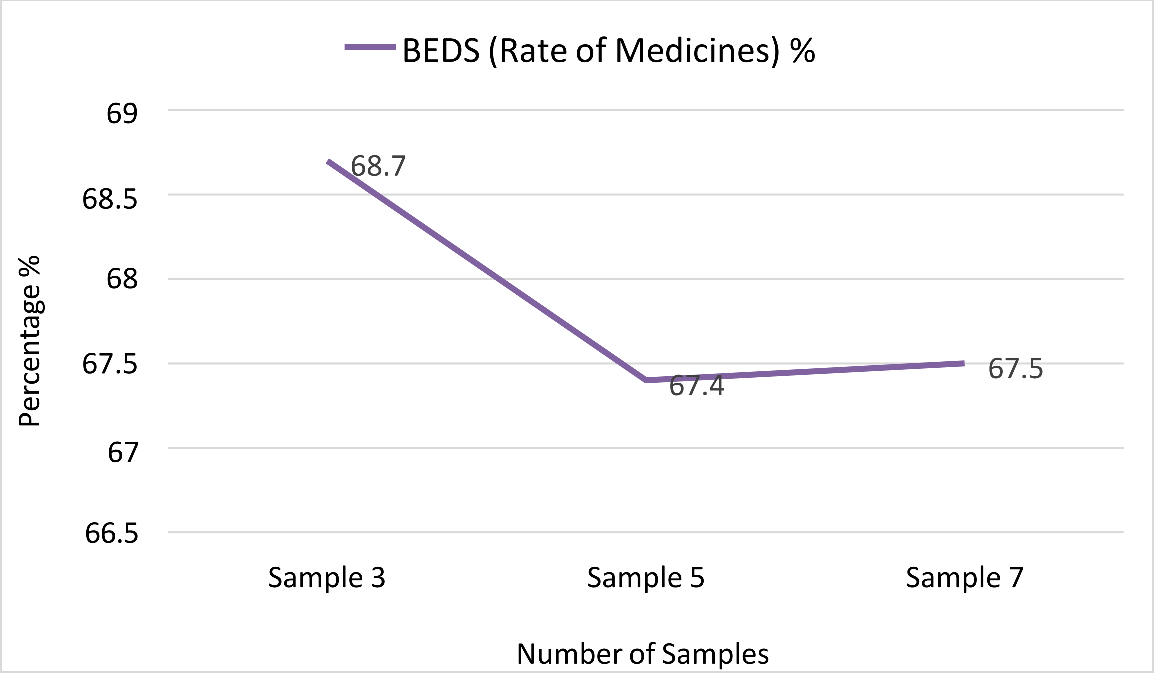

Figure 4.4 (b): concentration of pharmaceutical compound present in wastewaters (%) in system 2 (Beds) of Batch 1(1-15days) using HPLC

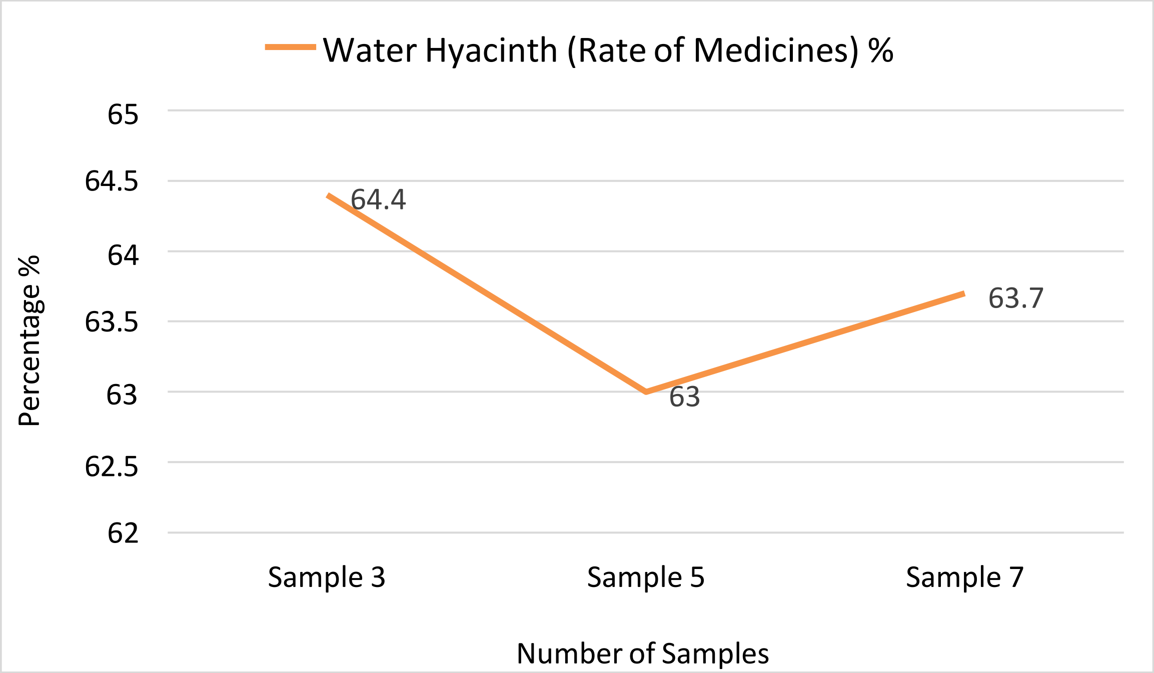

Figure 4.4 (c): concentration of pharmaceutical compound present in wastewaters (%) in system 3 (Water Hyacinth) of Batch 1(1-15days) using HPLC

4.4.2 Results of Batch 2(15-30 days)

In Batch 2, the Typha Australis system eliminated 37% of medicines from Sample 3, 32.3% from Sample 5, and 32.5% from Sample 7. The Water Hyacinth system attained removal rates of 30.5% for Sample 3, 30.8% for Sample 5, and 31.2% for Sample 7. The Beds system exhibited comparable removal efficiency, with 30% removal from Sample 3, 31.3% from Sample 5, and 32.6% from Sample 7. Overall, the Typha Australis system exhibited best results in this batch.

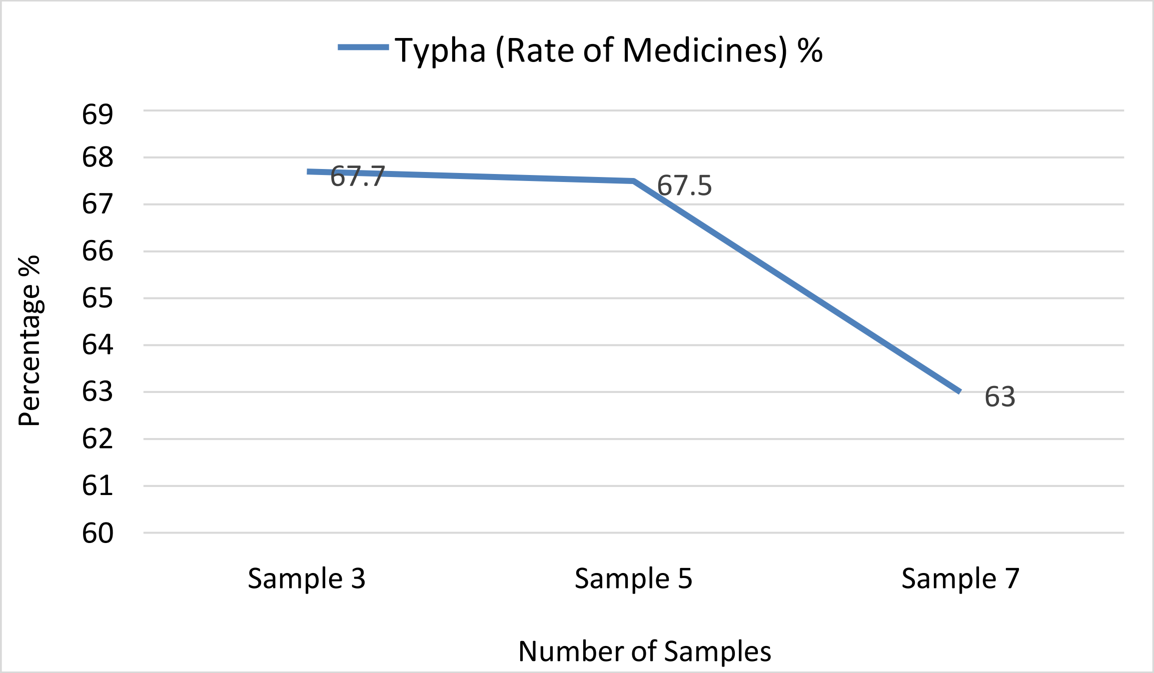

Figure 4.5 (a): concentration of pharmaceutical compound present in wastewaters (%) in system 1 (Typha Australis) of Batch 2(15-30days) using HPLC

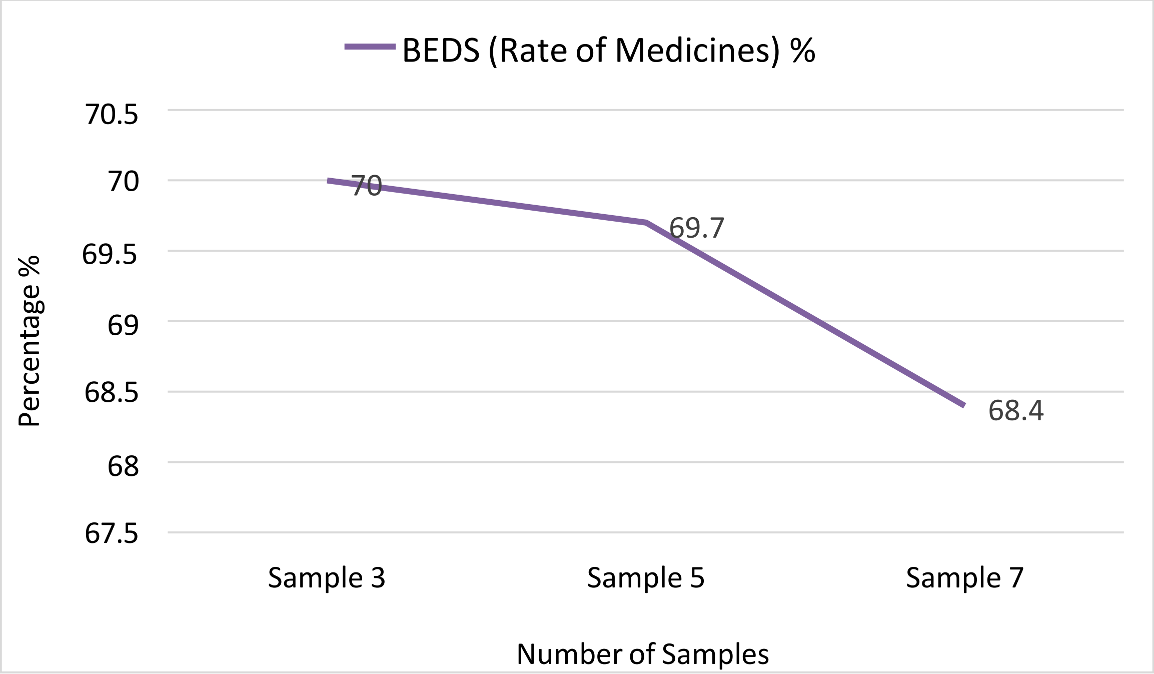

Figure 4.5 (b): concentration of pharmaceutical compound present in wastewaters (%) in system 2 (Beds)of Batch 2(15-30days) using HPLC

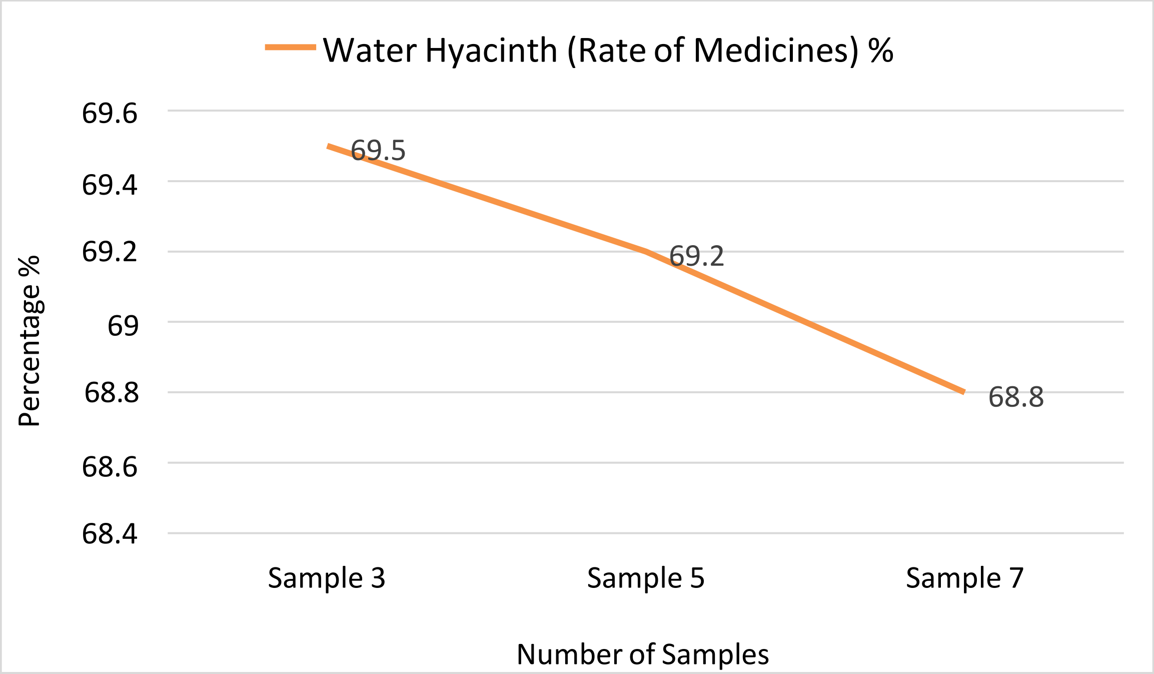

Figure 4.5 (c): concentration of pharmaceutical compound present in wastewaters (%) in system3 (Water Hyacinth) of Batch 2(15-30days) using HPLC

4.4.3 Results of Batch 3 (30-45 days)

In Batch 3, the Typha Australis system exhibited effective elimination rates of 34.5% for Sample 3, 34.9% for Sample 5, and 35.8% for Sample 7. The Water Hyacinth system demonstrated removal rates, reaching 30.1% for Sample 3, 33% for Sample 5, and 33.7% for Sample 7. The Beds system exhibited uniform removal rates, with 32.5% removed from Sample 3, 33.3% from Sample 5, and 32.6% from Sample 7. The results demonstrate that the Typha Australis system consistently exhibited best performance across all batches, whereas other two systems showed considerable efficacy in eliminating medications from the wastewater.

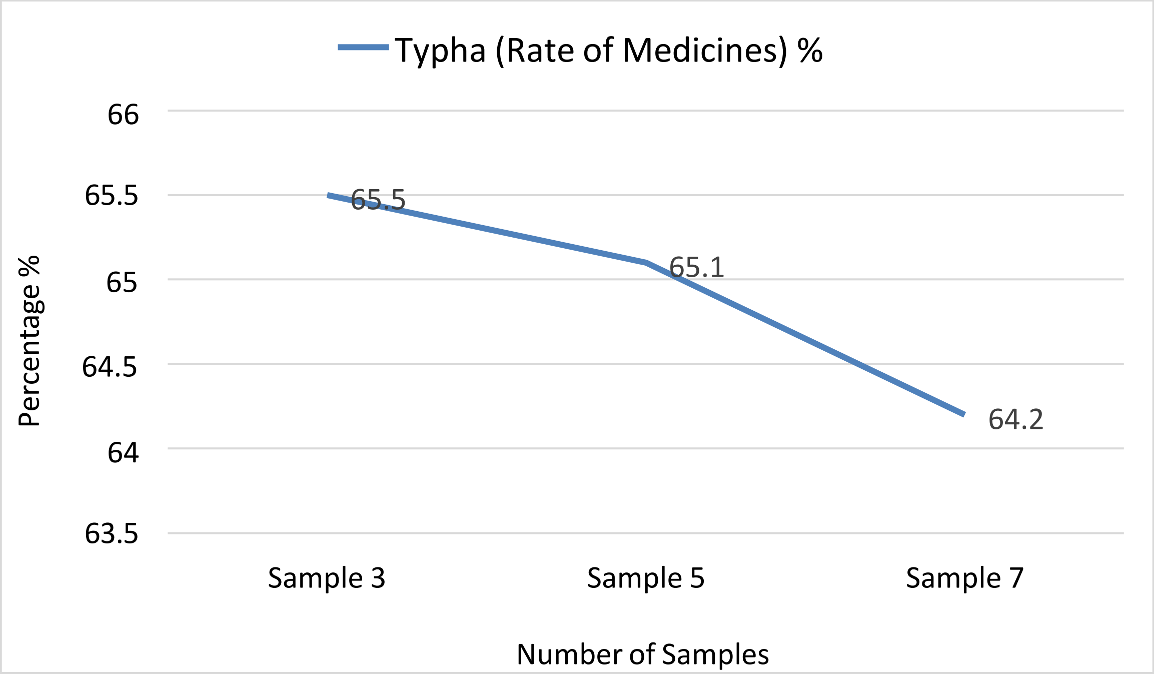

Figure 4.6 (a): concentration of pharmaceutical compound present in wastewaters (%) in system 1 (Typha Australis) of Batch 3(30-45days) using HPLC

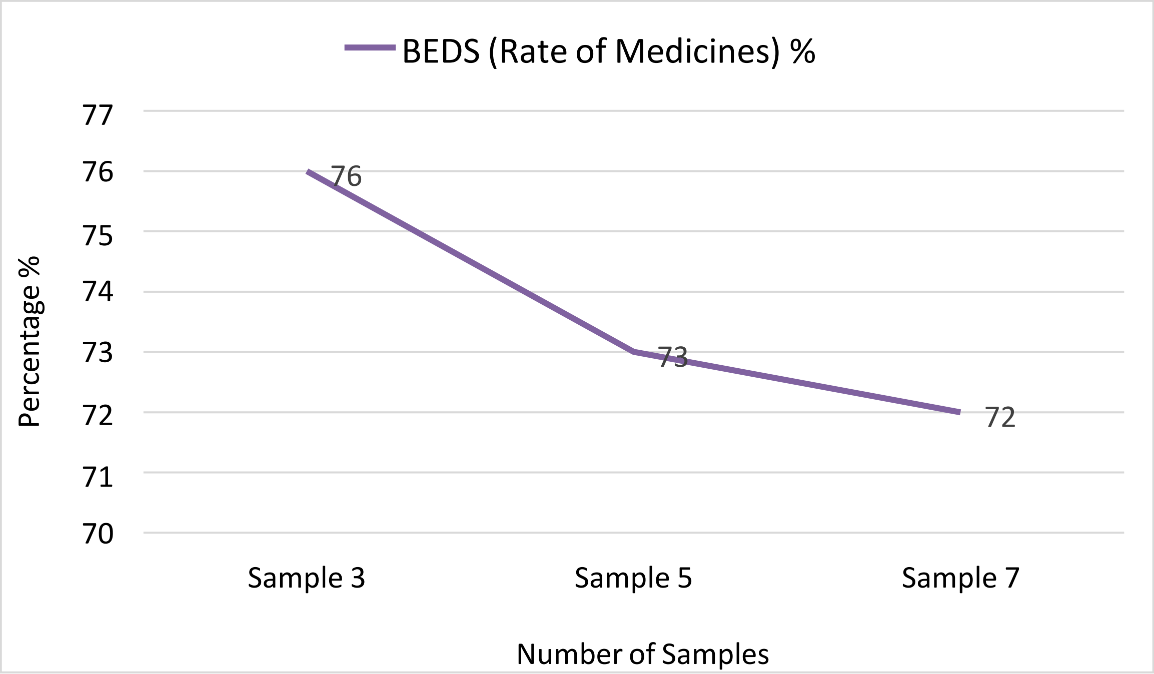

Figure 4.6 (b): concentration of pharmaceutical compound present in wastewaters (%) in system 2 (Beds)of Batch 2(30-45days) using HPLC

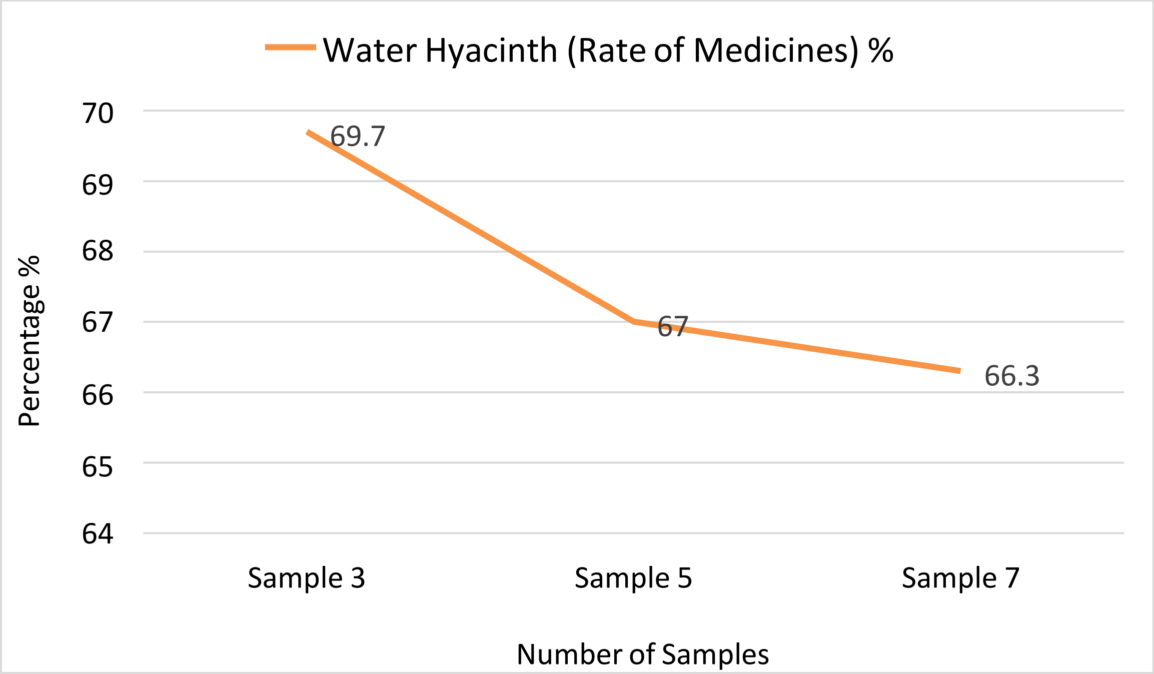

Figure 4.6 (c): concentration of pharmaceutical compound present in wastewaters (%) in system3 (Water Hyacinth) of Batch3(30-45days) using HPLC

DISCUSSION

This study investigated the efficiency of three constructed wetland systems, planted with *Typha Australis*, *Water Hyacinth*, and Beds, particularly in removing parameters such as COD and BOD and the pharmaceuticals, Ciprofloxacin, Cefixime, Ibuprofen, and Paracetamol across three experimental batches.

In Batch 1, the constructed wetland system with Typha Australis exhibited significant removal efficiencies, attaining a COD decrease from 257.1 mg/l to 88.3 mg/l, equating to a removal efficiency of 65.61%. BOD levels diminished from 218.3 mg/l to 71.7 mg/l, indicating an overall effectiveness of 67.1%. These substantial reductions demonstrate the system's ability to efficiently degrade organic contaminants, which is essential for enhancing water quality. The Water Hyacinth system exhibited effective performance in Batch 1, achieving a COD reduction from 283.1 mg/l to 156.9 mg/l (44.5% decrease) and a BOD reduction from 236.3 mg/l to 115.4 mg/l (51.4% reduction). Despite the removal efficiencies being lower to those obtained with Typha Australis, the findings still highlight the potential of Water Hyacinth in improving wastewater treatment. The Beds system demonstrated a decline in COD from 257.1 mg/l to 107.3 mg/l (58.2% reduction) and a decline in BOD from 218.3 mg/l to 94.6 mg/l (56.6% reduction). This performance demonstrated that the Beds system was effective in diminishing organic contaminants, however not to the same degree of efficiency as Typha Australis.

In Batch 2, the Typha Australis system exhibited similar results, with COD decreasing from 283.1 mg/l to 123.3 mg/l (a decrease of 56.5%) and BOD declining from 236.3 mg/l to 112.6 mg/l (a reduction of 52.3%). The uniformity in performance across batches indicated that the system is dependable and efficient in sustaining pollution removal over time. The Water Hyacinth system demonstrated robust performance in Batch 2, affirming its efficacy in decreasing COD and BOD levels. The Beds system showed considerable removal, with COD decreasing from 283.1 mg/l to 107.3 mg/l (62.1% decrease) and BOD from 236.3 mg/l to 94.6 mg/l (60.0% reduction). The comparable results among the three systems demonstrate that created wetlands are effective in wastewater treatment, irrespective of the plant employed.

In Batch 3, the Typha Australis system exhibited effectiveness, with COD levels declining from 296.4 mg/l to 156.9 mg/l (47.0% decrease) and BOD from 248 mg/l to 115.4 mg/l (53.4% reduction). Despite the removal efficiencies being somewhat lower than in prior batches, the system continued to efficiently diminish pollutant levels, thereby affirming its durability. The Water Hyacinth system continued to work effectively in Batch 3, with consistent reductions in both COD and BOD. The Beds system demonstrated a reduction in COD from 296.4 mg/l to 116.9 mg/l, representing a 60.5% decrease, and a reduction in BOD from 248 mg/l to 90.3 mg/l, indicating a 63.6% decrease.

The findings from all batches demonstrated that Typha Australis, Water Hyacinth, and Beds are proficient in diminishing BOD and COD levels in wastewater. The differing removal efficiency among batches may be ascribed to factors like the initial pollutant concentration, ambient circumstances, and the distinct properties of the constructed wetland systems. The reliable performance of these systems underscores their applicability in wastewater treatment, especially in areas where traditional approaches may be impractical. Constructed wetlands improve water quality and foster the sustainability of wetland ecosystems. The study emphasizes the significance of choosing suitable plant species for created wetlands to enhance the elimination of organic contaminants. The substantial decreases in BOD and COD seen in this study indicated the capability of these systems to comply with or beyond National Environmental Quality Standards (NEQS) for wastewater treatment, thereby aiding environmental conservation and public health. The comparative research of the three systems reveals that although Typha Australis may exhibit greater performance, both Water Hyacinth and Beds are viable solutions for sustainable wastewater management.

Utilizing High-Performance Liquid Chromatography (HPLC) analysis, the research also evaluated the removal rates of pharmaceuticals across three distinct batches. The results indicate that Typha Australis and Water Hyacinth were particularly effective in reducing pharmaceutical concentrations, with Water Hyacinth demonstrating the highest overall removal rates of pharmaceuticals. The pharmaceuticals removal rates (%) by each designed wetland system were determined based on the percentage of medications present in the wastewater. In batch 1 The Typha Australis system exhibited efficient removal from 43.5% to 42.9%. The Water Hyacinth system demonstrated higher removal rates, from 35.6% to 36.3%. Conversely, the Beds system exhibited decreasing removal efficiency, from 32.5% to 32.6%. The results showed that *Typha Australis* demonstrated highest average removal rates in Batch 1, where the other two systems had also shown considerable efficiency in eliminating medicines from the effluent. In Batch 2, the Typha Australis system exhibited removal rates from 32.3% to 37%. The Water Hyacinth system attained removal rates from 30.5% to 31.2%. The Beds system exhibited comparable removal efficiency, with 30 to 32.6%. In Batch 3, the Typha Australis system exhibited effective elimination rates from 34.5% to 35.8%. The Water Hyacinth system demonstrated removal rates, reaching 30.1% to 33.7%. The Beds system exhibited uniform removal rates from 32.5% to, 33.3%.

The findings highlighted that the constructed wetland systems not only improve water quality but also offer a sustainable approach to wastewater management. The consistent performance of these systems across different batches suggests their reliability and effectiveness in real-world applications. Furthermore, the research highlights the need for ongoing investigations into the long-term sustainability and operational efficiency of these systems. The comprehensive analysis demonstrated the importance of selecting appropriate plant species for constructed wetlands to optimize pharmaceutical removal.

The consistent performance of Typha Australis across all batches suggests its potential as a preferred choice for future wastewater treatment systems. Additionally, the study emphasized the need for further research into the mechanisms behind the removal processes, as well as the long-term sustainability and maintenance of these systems

CONCLUSION

This research evaluated the efficacy of constructed wetland systems, *Typha Australis, Water Hyacinth*, and Beds in treating synthetic wastewater, specifically in reducing COD and BOD and eliminating pharmaceuticals.

1. The study showed significant reductions with *Typha Australis*. In Batch 1, COD decreased to 88.3 mg/l (65.61% removal) and BOD to 71.7 mg/l (67.1% removal). Batch 2 reduced COD to 123.3 mg/l (56.5% reduction) and BOD to 112.6 mg/l (52.3% reduction). In Batch 3, COD levels fell to 156.9 mg/l (47.0% reduction) and BOD to 115.4 mg/l (53.4% reduction), enhancing the system's effectiveness.
2. The *Water Hyacinth* system also performed well in all batches. It reduced COD to 156.9 mg/l (44.5%) and BOD to 115.4 mg/l (51.4%) in Batch 1. Batch 2 lowered COD to 156.9 mg/l (44.5%) and BOD to 115.4 mg/l (51.9%). Batch 3 lowered COD to 156.9 mg/l (44.5%) and BOD to 115.4 mg/l (51.4%).
3. With comparatively lower reductions rates across all three batches, the Beds system showed effective treatment as well, Batch 1 lowered COD to 107.3 mg/l (58.2%) and BOD to 94.6 mg/l (56.6%). COD dropped 123.3 mg/l (55.6%) and BOD dropped 112.6 mg/l (52.10%) in batch 2 whereas in Batch 3 COD reduced to 172.3 mg/l (41.5%) and BOD to 122.6 mg/l (50.2%).
4. HPLC analysis highlighted the removal of pharmaceutical contaminants in three batches. The Typha Australis system exhibited best removal rate of 42.8%, Water Hyacinth system 36.3% and conversely, the Beds system exhibited 32.1% in batch 1. In Batch 2, the Typha Australis system eliminated an average of 33.9%, Water Hyacinth system 30.8% and the Beds system 31.3% pharmaceuticals. The Typha Australis system eliminated an average of 35%, Water Hyacinth removed 32.2% and Beds system eliminated 32.8% pharmaceuticals in Batch 3.
5. The *Typha Australis* system removed drugs with best removal rates while *Typha Australis* and beds systems also showed considerable results. The *Typha Australis* system also showed the best results in eliminating COD and BOD with greatest removal rates. The first batch had better treatment results than the other two.

RECOMMENDATIONS

Based on the key findings of the present study, the following recommendations are made for the future studies:

1. Due to the exceptional efficacy of Typha Australis, and Water Hyacinth in eliminating pharmaceuticals, it is recommended to give prominence to these species in the design and execution of artificial wetland systems for wastewater treatment.
2. The layout of synthetic wetlands must be specific to enhance the growth and vitality of chosen plant species. This encompasses factors such as water flow rates, substrate varieties, and managing nutrients to guarantee optimal plant growth and efficacy in pollution removal.
3. Implementing a systematic monitoring program to assess the efficiency of designed wetlands over time. This must encompass routine sampling and monitoring of water quality metrics (e.g., COD, BOD, pharmaceuticals) to guarantee that the systems consistently fulfill treatment objectives and regulatory requirements.
4. Examining the effects of seasonal variations on the efficacy of constructed wetlands. Comprehending the influence of temperature, precipitation, and plant development cycles on removal efficiencies can help guide management strategies and system layout.
5. Further research is necessary to evaluate the long-range viability of synthetic wetlands, focusing on the possible absorption of pharmaceuticals in plant tissues and the overall environmental impact of these systems.

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