DEVELOPMENT OF PLANTS AND MICROBES ASSISTED ENGINEERED WASTEWATER TREATMENT SYSTEM FOR EFFECTIVE REMOVAL OF ORGANIC CONTAMINANTS

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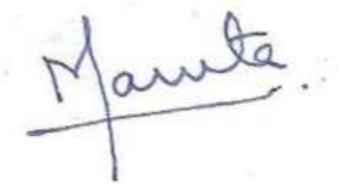
DECLARATION

I, hereby declared that the presented work in the thesis entitled “DEVELOPMENT OF PLANTS AND MICROBES ASSISTED ENGINEERED WASTEWATER TREATMENT SYSTEM FOR EFFECTIVE REMOVAL OF ORGANIC CONTAMINANTS” in fulfilment of degree of Doctor of Philosophy (Ph. D.) is outcome of research work carried out by me under the supervision of Dr Neeta Raj Sharma working as professor in the School of Bioengineering and Biosciences of Lovely Professional University, Punjab, India and Dr Rameshwar S Kanwar working as distinguished professor in Department of Agricultural and Biosystems Engineering, Iowa State University, Ames , USA. In keeping with the general practice of reporting scientific observations, due acknowledgments have been made whenever the work described here has been based on the findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "DEVELOPMENT OF PLANTS AND MICROBES ASSISTED ENGINEERED WASTEWATER TREATMENT SYSTEM FOR EFFECTIVE REMOVAL OF ORGANIC CONTAMINANTS" submitted in fulfillment of the requirement for the award of the degree of Doctor of Philosophy (Ph.D.) in the School of Bioengineering and Biosciences, Lovely Professional University, is a research work carried out by Mamta Sharma, bearing registration number 41800040 is a bonafide record of her original work carried out under my supervision and that no part of the thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Despite the availability of numerous efficient conventional wastewater treatment technologies, more than 80% of all the wastewater generated is released without adequate treatment into water bodies globally which not only degrades water quality and aquatic ecosystems but also has numerous negative implications on environment, human health and the overall well-being of communities. This issue is even more severe in middle and low-income countries that require simple and affordable alternative wastewater treatment technologies. Cost and the infrastructure involved in the traditional centralized wastewater treatment processes are the main bottlenecks, especially in densely populated developing countries. Conventional wastewater treatment methods are expensive and energy-intensive which require land, energy, infrastructure, and skilled manpower. Moreover, these processes produce secondary pollutants that need subsequent sustainable disposal. Therefore it becomes imperative to explore other ecological wastewater treatment solutions that are nature-based, sustainable, low-cost, and can be easily integrated with the existing wastewater treatment methods.

Constructed wetlands (CWs) are one such ecologically engineered and self-adaptive wastewater treatment and management systems that are designed to employ the processes taking place in a natural wetland to treat different kinds of wastewater with a greater degree of control and accuracy. It is a comparatively inexpensive and promising technology that can be a suitable alternative to the technologically complex and expensive conventional wastewater treatment processes. Constructed wetlands are extremely versatile systems in terms of their design, kind of media used, variety of plant species grown and the type of wastewater being treated. Over the past few decades, constructed wetlands have been established as an efficient natural solution for wastewater treatment that offers numerous additional ecological benefits like groundwater recharge, carbon sequestration, recreational uses, wildlife habitat, aquaculture, flood control, and improved aesthetic value in addition to treating wastewater.

Constructed wetland treatment systems (CWTS) comprise three main components: i) substrate (media), ii) microbes, and iii) plants. For effective wastewater treatment using constructed wetlands, the choice of media and plant species is crucial. Most of the constructed wetland studies are carried out using weeds like *Phragmites australis.* Even today the majority of studies use *Phragmites australis, Typha, Schoenoplectus*, etc. which is not a very good choice due to its invasiveness and less commercial value. Thus, the present study was undertaken to explore the tolerance of four ornamental plants (*Canna indica, Gerbera jamesonii, Lilium wallichianum,* and *Tagetes erecta*) under wastewater stress conditions. The use of ornamental plants in the constructed wetlands is an attractive option not only owing to its aesthetic and commercial value but also because of the other added benefits that include ecosystem services, biodiversity enhancement, revenue generation opportunities and easy integration of these systems with rural and urban landscapes.

Conventionally, soil, gravel, and sand have been used as substrates in constructed wetlands. The incorporation of various natural and artificial materials as components of wetland media has been investigated over the years; however, the influence of agricultural waste (crop residues) as wetland media, on the treatment efficiency of the system is rarely documented. Few recent studies have reported that agricultural residues and biochar produced from them can be used as additives or substitutes for conventional plant-growing media. The state of Punjab is one of the major agricultural growing areas in India. Some of the important crops grown in Punjab are rice, wheat, and sugarcane which results in surplus crop residues in the field after harvest. To get ready for the next plant growing season, farmers prefer to burn these excess crop residues in the field rather than mulching them into the soil with a tillage tool due to a lack of economically affordable technologies. Burning of surplus residues in agricultural fields is a common practice in many parts of the world. This practice adds emissions into the atmosphere and results in the loss of essential plant nutrients; hence, there is a need for developing technologies for the sustainable management of agri-residues.

Rice straws left in the fields and sugarcane bagasse discarded by sugarcane factories after extraction of juice are the two most abundantly available agricultural by-products in Punjab. Utilizing these locally available low-cost biomaterial resources as substrates in constructed wetlands, will not only help with its disposal and mitigating with its negative environmental impacts but will also greatly reduce the overall cost of the constructed wetland system. For the selection of substrates in the present study, abundantly available low-cost agricultural wastes in the region were explored in six different treatment combinations. Incorporation of these organic materials as substrates in CWTS will improve the filter bed properties mainly by increasing the carbon source and enhancing the surface adsorption of pollutants on the media components. In fact, building these systems using locally available media and native plants will make these systems more cost-efficient as well as better suited to the conditions and requirements of a specific region. The present study is an attempt to use locally available surplus organic materials as media components to grow native ornamental plants in mesocosm wetland systems to make these systems more cost-effective, profitable, and easily adoptable by individuals, rural and urban communities, organizations, and industries.

In the present study, firstly, the use of rice straw, sugarcane bagasse, and biochar as substrates to grow ornamental plants in constructed wetlands was investigated. Four ornamental plants were grown in six different substrate combinations (T1-T6) for 120 days. Data on plant growth parameters were collected for each plant and compared to select the best substrate combination. *Canna indica* and *Lilium wallichianum* resulted in significantly higher growth and nutrient uptake ($P<0.001$) with the substrate of 15% rice straw, 80% soil, 5% biochar (T4), and 25% sugarcane bagasse, 70% soil, 5% biochar (T5) compared to other plants. Further, the potential of these vertical sub-surface flow mesocosm-constructed wetland systems for wastewater treatment was evaluated. Based on plant growth observations, *Canna indica, Lilium wallichianum,* and *Tagetes erecta* were selected to be grown in three media namely soil and biochar (M1); soil, biochar, and rice straw (M2); soil, biochar, and sugarcane bagasse (M3). The influent and effluent were analyzed for pH, TDS, organic matter and dissolved oxygen demand (BOD and COD), phosphorus (PO_4-P), and nitrogen forms i.e. ammonia (NH_4-N) and nitrate (NO_3-N) using standard methods for five weeks. Lastly, various important design aspects that influence the pollutant removal efficiency of constructed wetland treatment systems were identified and key considerations during each stage to achieve optimum performance are proposed.

Investigated mesocosms showed an average removal efficiency of 49.21% for BOD, 53.76% for COD, 40.64% for NH_4-N, 41.76% for NO_3-N, and 21.53% for PO_4-P. The result shows that the designed mesocosms are a promising nature-based alternative to the conventional centralized wastewater treatment technologies, with numerous additional ecological benefits. The study concludes that agricultural waste-derived substrates are viable alternatives having fertilizing effects with the potential for nutrient recovery. After their use in wetlands; these digested organic materials may further be used as an effective source of nutrient-rich fertilizers, or soil amendments in agriculture and as a source of carbon to mitigate climate change. The present study provides an alternative approach to utilize agricultural waste sustainably to grow ornamental plants in the constructed wetland which reduces the overall cost of the wetland unit making it more cost-efficient.

Various other abundantly available agricultural wastes can also be considered and evaluated for their potential for plant growth and pollutant removal efficiency, but future studies are needed in testing and evaluating those materials. Future work can also be targeted toward utilizing the saturated substrates for their fertilizer value. Techniques to recover valuable plant nutrients from the saturated substrates are another potential area to be investigated further. Future studies can also focus on evaluating the influence of CW design and operational parameters such as wastewater loading rates at different concentration of pollutants, varying retention rates for different types of media, using other plants compared to ornamental plants used in this study, and promoting CW as a climate mitigation and environmentally friendly practice for treating wastewater which is a major problem faced by the global community.

Affectionately Dedicated

To

"Anaisha"

my blessing, my pride, my joy

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Mamta Sharma

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ABBREVIATIONS

CW	Constructed Wetland
ANOVA	Analysis of variance
APHA	American public health association
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CPCB	Central pollution control board
CW	Constructed wetland
CWTS	Constructed wetland treatment system
DBT	Department of biotechnology
EC	Electrical conductivity
ET	Evapotranspiration
GDP	Gross domestic product
HLR	Hydraulic loading rate
HRT	Hydraulic retention time
IARI	Indian agricultural research institute
LPU	Lovely professional university
MLD	million litres per day
MNRE	Ministry of new and renewable energy
NGT	National green tribunal
NH_4-N	Ammonia
NITI	National institution for transforming India
NO_3-N	Nitrate
NPMCR	National policy for management of crop residues
PO_4-P	Phosphorus
ppm	Parts per million
ROI	Return on investment
SDG	Sustainable development goal
SPAD	Soil plant analysis development chlorophyll
STP	Sewage treatment plant

TDS	Total dissolved solids
TKN	Total kjeldahl nitrogen
TSS	Total suspended solids
UN	United Nations
UNEP	United Nations Environment Programme
NEERI	National Environmental Engineering Research Institute
SWAB	Scientific wetland with active biodegradation
SWINGS	Safeguarding Water resources in India with Green and Sustainable technologies

Chapter-1

Introduction

1.1. Wastewater: a growing global concern

Water is one of the most imperative and critical resource for existence of life, but still a third of the global population is living in water stressed conditions (UNEP, 2023). Rapid urbanization, industrialization, fast paced socio-economic development and rampant population are placing a huge strain on the resources at a vigorous rate (Sharma and Sharma, 2020). Decades of overconsumption, improper handling of wastewater, insufficient recycling, and mismanagement of the finite water resources with intensified water demand have made the available water scarcer and polluted resulting in global water crisis. Parallelly, climate change is adding to the shortage of water availability due to extreme floods and droughts (NITI, 2022). Unhygienic water bodies, improper water distribution, insufficient wastewater collection, inadequate treatment facilities and negligence in reusing treated wastewater is further amplify the problem of water scarcity.

World's population is estimated to be almost 10 billion by 2050 which is expected to lead to an alarming increase in the amount of wastewater being produced. In 2013, the estimated production of wastewater from municipal sources was 330 billion m^3/year which increased to 360–380 billion m^3/year by 2015. By 2030, it is further estimated to rise to 470–497 billion m^3/year (UNEP, 2023). Thus it is necessary to manage the available water efficiently to enhance the quality of life, improve environmental health, to support socio-economic growth. Developing efficient and economical wastewater treatment technologies that enable reducing, reusing and recycling wastewater can be a valuable contribution towards sustainable development and circular economy (Shanmugam et al., 2022).

1.2. India's wastewater scenario: at a glance

India accounts for 16% of world's population and only 4% of world's water resources. The estimated wastewater generation in India with a population of 1.38 billion people, for rural and urban centers was 39,604 million litres per day (MLD) and 72,368 MLD respectively for the year 2020-21 (NITI, 2022). Planning commission has estimated 2.5 times increase in domestic and industrial water consumption by 2050 (CPCB, 2008). There is a considerable gap in the amount of wastewater generated and capacity of the existing sewage treatment plants. As per CPCB 2016 report, only 37% of the sewage generated is treated rest 63% of the sewage remains untreated and is directly discharged into water bodies. Few states (7) do not have a single sewage treatment plant (CPCB, Bulletin 2016). Apart from this, non-compliance with discharge standards and unregulated discharge of industrial effluents is another serious concern. The existing sewage treatment capacity also remain underutilized in a lot of places dues to operational and maintenance issues. Many sewage treatment facilities fail to meet the necessary effluent requirements.

Central Pollution Control Board (CPCB) evaluated the performance of 152 sewage treatment plants (STPs) of 15 states in the country and reported only 66% actual treatment capacity utilization. Out of 152 treatment plants some were non operational (30), few under construction (9), effluent of some plants exceeded BOD (49) and COD (7) limits and performance of some was not satisfactory (28). Wastewater treatment capacity of metropolitan cities, class-I cities and class-II towns was reported to be 51%, 32% and 8% respectively. The capital cost for treatment of one million liter per day (MLD) wastewater ranges from 0.63 Crore to 3 Crore with additional operation and maintenance cost of around Rs. 30,000 per month and the total sewage generated in metropolitan cities, class I cities and class II towns was nearly 53,899 MLD (CPCB, 2013). National green tribunal and Honourable Supreme Court have directed to examine best possible technologies to bridge the huge gap in the sewage generation and treatment capacity.

1.3. Wastewater: risks and related implications

Despite the availability of numerous efficient conventional and advanced wastewater treatment technologies, globally, more than 80% of all the wastewater generated is released without adequate treatment which not only degrades water bodies and aquatic ecosystems but also has numerous negative implications on human health and the overall well-being of communities (UN-Water, 2017). Almost 80% of the water supplied for domestic use re-enters the system as wastewater (CPCB, 2013). The uncontrolled and unregulated disposal of untreated or partially treated wastewater deteriorates water quality and has hazardous impact on human and environmental health (Obaideen et al., 2022).

Discharging untreated or poorly treated wastewater result in addition of various contaminants into surface water bodies every day that not only pollutes the receiving body but also causes number of diseases in people coming directly or indirectly in contact with the contaminated water. Presence of bacteria, viruses and parasitic pathogens can cause mild to serious waterborne illnesses like cholera, typhoid fever, dysentery, hepatitis A, giardiasis, cryptosporidiosis and other gastrointestinal track issues like diarrhea, vomiting, nausea and stomach cramps. Additionally, presence of pathogens is also contributing towards the spread of antibiotic-resistant bacteria which is another major issue in managing infections. Apart from this, contact with polluted water can also cause dermatitis, skin irritation, rashes and other skin conditions. Long term exposure to contaminants including heavy metals, chemicals, and persistent organic pollutants present in wastewater is related to cancer, neurological disorders and organ damage.

Wastewater can contain diverse range of contaminants like organic matter, heavy metals, pathogens, chemicals etc. These contaminants can dissolve or remains suspended in the water bodies. Some pollutants get deposited on the bed of the water body and some can sweep down and pollute groundwater reserves. Nutrients like nitrogen, phosphorus and organic matter present in wastewater serves as food for microbes. The addition of excess nutrients, particularly nitrogen and phosphorus, promotes the overgrowth of algae, microbes, and plants in water bodies, a process called eutrophication. High nitrogen levels in wastewater can cause algal blooms which can create toxins. Moreover, decomposition of dead plant biomass by microbes reduces dissolved oxygen of the water body and creates a state of hypoxia. This damages aquatic life forms, creates ecological imbalances and impairs the health of ecosystems.

1.4. Conventional wastewater treatment approaches

Most traditional approaches for wastewater treatment utilizes a combination of various physical and chemical methods like sedimentation, sand filtration, membrane separation, coagulation, flocculation, adsorption, chemical precipitation, etc to remove contaminants from wastewater. Some superior methods include advanced oxidation, ion-exchange, solvent extraction, ultrafiltration, electrodialysis, etc (Crini and Lichtfouse, 2019). The majority of sewage treatment plants are based on physicochemical and anaerobic processes or a combination of both. Trickling filters, high-rate stabilization ponds, maturation ponds, anaerobic ponds, anaerobic sludge blanket reactors, etc are also employed in many places to treat wastewater (Singh et al., 2019). Frequent issues encountered with most of the processes are high initial capital cost, energy and electricity costs, manpower costs, chemical consumption, maintenance, and operational costs, equipment corrosion, and high sludge generation which need further treatment and disposal (Fig. 1.1) (Crini and Lichtfouse, 2019; Singh et al., 2019).

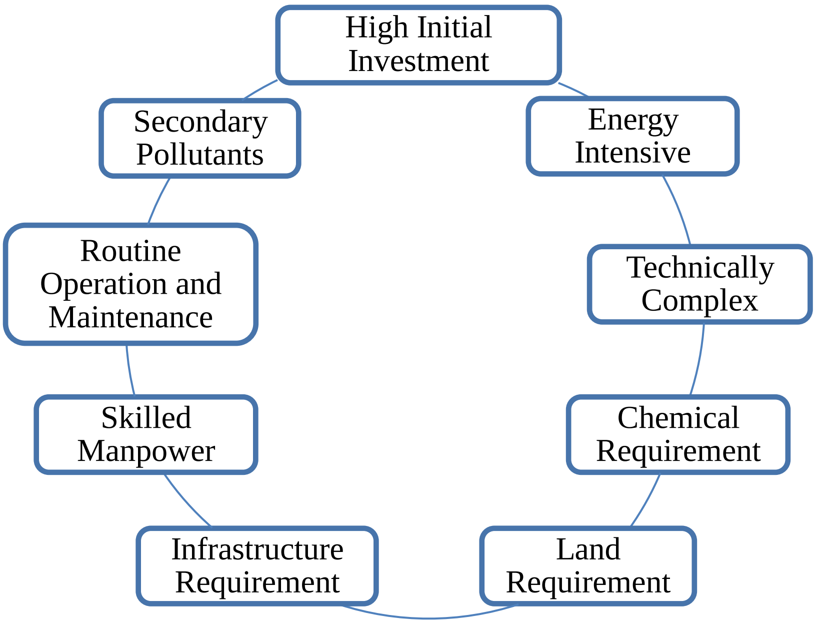


Fig 1.1 Limitations of conventional wastewater treatment methods

1.5. Challenges in managing wastewater

Even today, the primary treatment of wastewater remains a challenge in many parts of the world (UNEP, 2023). Understanding and addressing the challenges involved in wastewater treatment is crucial to achieve sustainable wastewater management. Various issues encountered in wastewater management are listed in table 1.1. Firstly, cost and the infrastructure involved in the traditional centralized wastewater treatment processes are the main bottlenecks, especially in densely populated developing countries. Secondly, the rate at which the demand for clean water is increasing is at a much faster pace than the technological developments in the wastewater treatment solutions. Employing advanced pollutant removal technologies further increases the overall cost of the treatment. Maintaining maximum treatment efficiency of the existing sewage treatment plants and installing new treatment plants needs good financial assistance.

In developing countries many regions do not have well developed sewage collection systems that are connected to a wastewater treatment plant. This is mainly due to low investment in the wastewater collection and treatment infrastructure. Conventional wastewater treatment methods are expensive and energy-intensive which require land, infrastructure, and skilled manpower. Besides, these processes produce secondary pollutants that need subsequent sustainable disposal. Apart from the huge initial setup and installation cost, normal operation, maintenance, repair, chemical cost, and quality assessment also add to the overall expenses. Another major challenge is the presence of complex and heterogeneous pollutants that require highly efficient and advanced hybrid system which are usually expensive. Due to the diverse nature of the contaminants present in the polluted water, there cannot be a universal technique to treat all kinds of wastewater (UNEP, 2023).

Other limitation includes topography of areas like mountainous terrains, places with reduced land availability and difficulty in accessibility of the site. In many locations there is absence of a power supply unit on site to set up a conventional treatment plant. Furthermore, there is a serious lack of awareness about reusing treated water, for instance, only 11% of the total wastewater produced from domestic and industrial sources is presently being reused. In France, only 0.1 % of the treated wastewater is reused (UNEP, 2023).

Table 1.1 Challenges in wastewater treatment

Various obstructions faced in efficient treatment and management of wastewater
High cost involved in setting up a treatment plant
Lack of required infrastructure
Electricity and chemical requirements
Regular operation, maintenance, troubleshooting, record keeping, quality control

requires cost and manpower
Smaller/ rural communities where land is available and is relatively inexpensive but finding skilled operators are difficult
Lack of sustainable sludge disposal methods and associated cost
Missing ownership and lack of social responsibility
Complex and heterogeneous nature of pollutants
Lack of proper regulations
Lack of productive reuse of treated wastewater
Inefficiency in removing emerging contaminant
Governance, institutional and regulatory barriers
Low levels of implementation, with weak compliance and enforcement
Lack of priority setting and support in the political arena
Inadequate financing and cost recovery initiatives
Lack of revenue generation opportunities
Low social and cultural acceptance
Limited skill, manpower and institutional capacity
Negative perceptions associated with wastewater
Underutilization of existing treatment facilities

It is important to understand all challenges to design efficient and economical solutions for management of wastewater. Efficient and affordable technologies need to be designed with the intention of reusing the treated water. Recycling and reusing treated water will reduce the dependence on rainfall and overexploitation of groundwater reserves. Efforts should be directed not only in providing clean water but focus should also be on treating the huge amount of wastewater generated in a sustainable manner and reusing it for other purposes like gardening, irrigation, washing etc (Singh et al., 2019). Various innovative solutions have been developed over the years but it's important to accelerate its pace and facilitate actual implementation on field. Establishing efficient wastewater management and treatment solutions is utmost important to achieve sustainable development.

1.6. Role of wastewater management in achieving the SDGs

Water is a key resource that on one hand acts as catalyze for growth and on the other hand holds the potential to hinder the development if not managed sensibly. Wastewater management directly or indirectly plays important role in achieving 11 out of 17 sustainable development goals (SDGs). Efficient treatment of wastewater process increase water availability thereby directly contributes towards SDG6: clean water and sanitation. Wastewater management is central theme in many other SDGs as well, for instance, wastewater treatments technologies help to improve water quality hence reducing potential diseases, cutting down the wastewater discharge in natural water bodies whereby minimizing its negative impacts on the marine life (SDG3: good health and well-being and SDG 14: life below water).

Improving water management system helps to reduce the environmental impact of wastewater, improves waste circulating process and recycling water for agricultural and other purposes (SDG 2: zero hunger, SDG 12: responsible consumption and production, SDG 11: sustainable cities and communities and SDG 13: climate action). Apart from providing a new income source to smallholders, creating new jobs and revenue streams; water management processes also helps to reduce the price of clean water used in all production processes thereby increasing gross domestic product (GDP) (SDG1: no poverty and SDG 8: decent work and economic growth). Additionally, management of wastewater provides innovative opportunities to develop business models for recovery of valuable products (SDG 9: industry, innovation and infrastructure) thereby adding value to the wastes and generation of biogas and energy from biomass (SDG 7: affordable and clean energy) (Obaideen et al. 2022). More efforts need to be directed to recognize wastewater as a circular economy opportunity.

1.7. Wastewater the "untapped" and "undervalued" resource

Wastewater has the potential to supply around 320 billion cubic meters per year water for reuse; it's more than 10 times the present global desalination capacity (UNEP, 2023). It can also be used to replenish depleted groundwater resources. Agriculture takes around 70% of freshwater withdrawals. Reusing treated water for irrigation purposes will substantially reduce dependence on rainfall and other fresh water sources. Apart from agriculture, reclaimed water can also be utilized for landscape irrigation purposes, like in parks, gardens, golf courses and other green spaces. In industries, it can be used for cooling and cleaning purposes.

Various resources that can be recovered from wastewater are shown in Fig. 1.2. It is an absolute priority to efficiently manage the wastewater along with recognizing the inherent value of wastewater and harnessing its underutilized potential. There is need to transform the perception of wastewater from a waste management issue and being a source of pollution to a flourishing valued resource for clean water, nutrients and energy (UNEP, 2023).

Water	**Nutrients**	**Energy**
• Increased water security	• Reduced dependance on synthetic fertilizers	• Diversified energy production (electricity)
• Potential of irrigate millions of hectares of land	• Soil amandement	• Capitalising on the opportunities for resource recovery
• Reduced dependence on rainfall	• Economic opportunity	• Increased energy security

Fig.1.2 Wastewater as resource for clean water, nutrients and energy

1.8. The way forward: nature based solutions

For several decades, researchers have been exploring various nature-based solutions that are economical alternatives to conventional wastewater management systems which are financially expensive as well as technologically complex. Natural wetlands are one such water-saturated ecosystem comprised of biotic and abiotic components which use plants, microbes, and their interactions for removal of contaminants. Wetlands are also referred to as “kidneys of the landscape” and “biological supermarkets”. Being reservoir of food and shelter it provides habitat to diverse biological communities (Gupta et al., 2020; DBT-CPCB Manual, 2019). Natural wetlands are well adapted to water logging and restricted aeration conditions. Use of natural wetlands as convenient recipient of wastewater discharge is an old practice. Some wetlands have been used as wastewater discharge sites for centuries

(Vymazal, 2011). Harnessing potential of plants and microbes to treat wastewater has minimal energy requirements, less operational and maintenance efforts, and generates zero residues (Singh et al., 2019).

1.9. Constructed wetlands

Constructed wetlands (CWs) are engineered systems that are designed to mimic processes taking place in natural wetlands i.e. utilize bioremediation and phytoremediation for the transformation and removal of pollutants from wastewater. Constructed wetlands are efficient green technology based on the natural systems and ability of plants and microbes to remove various types of pollutants. These artificial systems have been explored for their potential to treat wastewater since 1960. These systems offer the advantage of being operated in more controlled environments (Vymazal, 2018).

Nature-based solutions like constructed wetlands support the wider vision of a circular economy not only by offering sustainable management of wastewater but also by providing numerous additional ecosystem services and social benefits (Stefanakis et al., 2021). Constructed wetlands appear to be an ideal option for wastewater management and are promising alternatives in the field of decentralized wastewater treatment (Stefanakis, 2019). Simple construction and low maintenance of constructed wetlands makes them an attractive option for both rural and urban settings (DBT-CBCB Manual, 2019). In addition to removing pollutants, such systems are aesthetically pleasing and enhance the landscape. Such systems can be adopted by large units like industries, municipalities as well as by smaller communities like panchayats and even individuals.

Media or substrate, plants, and microbes are the principal components of constructed wetlands (Vymazal, 2020; Vymazal, 2022). For effective wastewater treatment using CWs, the choice of substrate and plant species is crucial. Plants play a significant role in wetland treatment systems. The presence of plants in the system significantly increases its efficiency. Plants in the wetland system not only enhance the wildlife habitat value and aesthetic appearance but stabilizes the sediments, support the physical structure, and enhance the transformation of contaminants in the rhizosphere (Tanner, 2001). The mechanical action of plants helps to prevent the clogging of the deposited matter. It also helps in stabilizing and holding the matrix together (Latune et al., 2017). For the selection of appropriate plant species, it is important to understand plant resilience and monitor its adaptation to wastewater stress conditions. The ability of the plant to survive in the flooded condition, the nutrient load, and its tolerance to the variability and toxic effects of the wastewater are important parameters (Li et al., 2013).

The substrate which is also known as media, filling material, support matrix, or filter bed is another major component of the wetland system through which wastewater is made to flow. Substrates play a significant role in the removal of pollutants by supporting plant growth and providing a surface for biofilm growth around the media particles. Most of the biological, chemical and physical processes take place in the substrate which makes the wetland healthy and biologically functional (Vymazal, 2022; Wang Y et al., 2020).

The study attempts to evaluate the utilization of agricultural residue materials as substrate for the growth of ornamental plants in a constructed wetland system. It is hypothesized that the incorporation of agricultural residues in the substrate will promote the growth and flowering of ornamental plants. To test the hypothesis, rice straw, sugarcane bagasse, and biochar were used as part of the substrate in a pot study. The growth characteristics of *Canna indica*, *Gerbera jamesonii*, *Lilium wallichianum*, and *Tagetes erecta* were evaluated in six different substrate treatment combinations for a period of 120 days. Rice straw, sugarcane bagasse, soil, and biochar were combined in different ratios to create six treatment combinations. Plant height, number of leaves, SPAD unit, number of flowers, days to flower emergence, stem thickness, microscopic examination, root morphology, biomass, and elemental analysis of leaves were observed and compared for the selection of the best substrate combination for each plant. Further, the efficiency of the selected substrate and plants were investigated for pollutant removal.

Chapter 2
Review of Literature

2.1. Constructed wetlands for wastewater treatment

Constructed wetlands (CWs) are ecologically engineered and self-adaptive systems that are designed to employ the natural process taking place in a wetland to treat different kinds of wastewater with a greater degree of control (Ghosh and Gopal, 2010). It is a comparatively inexpensive and promising technology that can be a suitable alternative to complex conventional and costly advanced wastewater treatment processes (Ajibade et al., 2021). From the early 1950s when the first wetland study was carried till present time CWs have been extensively employed to treat various different types of wastewater. Over the past few decades, constructed wetlands has firmly established its roots and is recognized as an efficient natural solution for wastewater remediation and an excellent alternative to the conventional treatment processes (Ghosh and Gopal, 2010). Additionally, it offers numerous other ecological benefits like groundwater recharge, carbon sequestration, recreational uses, wildlife habitat, aquaculture, flood control, and add aesthetic value apart from treating wastewater (Kumar and Dutta, 2019). The development of constructed wetland treatment technology over the years and its potential as alternative wastewater treatment and management technology is reviewed. Various components of the system, their role and processes involved in removal of contaminants are also discussed. Emphasis is given on selection of suitable plants and benefits of incorporation of agricultural wastes as substrates. Comprehensive review of various plants and substrates used in constructed wetlands and their influence on removal of different types of pollutants is also presented. Lastly, advantages and limitations of the constructed wetland treatment systems (CWTS) are discussed.

2.2.Constructed wetlands early development and evolution

In early 1950s small scale systems using wetland plants were examined for their ability to treat wastewater (Gupta et al., 2020; Vymazal, 2011). The initial attempt of treating wastewater using wetland plants was done by Dr. Kathe Seidel in the early 1960s at Max Planck Institute, Germany. In Dr. Kathe’s hydrobotanical systems wastewater was made to flow through a series of sand and gravel beds having emergent wetland growing on them. Efficient removal of BOD, TSS, N, and P was reported in the initial studies. The first full scale system using wetland plants developed by Dr Kathe Seidel became operational in late 1960s in Germany (Vymazal, 2010). She proposed that using porous media had high hydraulic conductivity as a substrate. Many attempts thereafter were made to grow the emergent plants in wastewater and sludge (Vymazal, 2005).

Many improvements in the root zone method were done by Reinhold Kickuth in the 1970s. In early designs, horizontal sub-surface flow CW planted with common reed was the most common type of wetlands used. Roots of the plant were used to penetrate through the bed and release oxygen in the root zones which facilitated aerobic degradation of contaminants. Later studies showed that little oxygen was available in the zone and anoxic and anaerobic decomposition are also important aspects of the treatment process (Vymazal, 2005). Johansen and Brix introduced hybrid wetland systems in the mid-1990s in which water was made to flow through horizontal and vertical beds. Vymazal (2010) suggested employing hybrid systems i.e. combination of different CW designs to achieve enhanced removal of nutrients.

From 1970s to 1980s most of the artificial wetland systems were used to treat domestic and municipal wastewater in many different countries like in Hungary FWSCW was developed to treat town wastewater in 1968. Around this time North America also started using these systems for treatment of all types of wastewaters. In North Dakota, Amoco Oil Company’s Mandan Refinery CW was set up for the treatment of industrial storm water and process water in the year 1975. In 1977 in Othfresen, Germany, the first full scale CW for the treatment of municipal sewage was developed. In 1980s in California, CWs were used for treatment of urban wastewater. By 1990 more than 500 of such reed beds or root zone systems were operational in different parts of the world like Europe, USA, Norway, China and Canada. These systems were used to treat all kinds of wastewater like domestic, municipal, agricultural, dairy, landfill leachate, food processing, mine drainage and many more (DBT-CPCB Manual, 2019). United States Environmental Protection Agency in 1980s had given consent to employ CW for treatment of domestic wastewater. With time these systems became popular and were used in different parts of the world. Many filter beds using different types of wetland plants were operational in different parts of Europe, North America and Australia. Majority of these systems were planted with *Phragmites australis* i.e. common reed. Therefore these systems were also commonly referred to as “Reed beds” or “Reed bed treatment system” in Europe and United Kingdom.

Constructed wetlands have evolved over several decades as a sustainable approach for wastewater management and treatment. Early experiments in mid-20th century were more focused at comprehending the natural processes that wetlands provide. The ecological services provided by wetlands, such as their natural capacity to filter and treat water, came to the attention of more people in the 1960s and 1970s. Various organizations, scholar and environmentalists started advocating for protection and use of natural and artificial wetlands for water management. In the 1970s and 1980s, constructed wetlands began to be utilized for wastewater treatment. These early initiatives tested the viability of using wetlands to treat different kinds of wastewater and were frequently small-scale and experimental.

Wetland design as well as technology advanced throughout the 1980s and 1990s as interest in constructed wetlands increased. The structure of wetland cells, plant species selection, and hydraulic issues were the main areas of interest for the researchers. Governments and environmental organizations began to recognize the potential of constructed wetlands for long-term, sustainable wastewater treatment, in the 1990s and early 2000s. To facilitate the incorporation of these systems into existing conventional technologies, guidelines and regulations were developed. The 21st century has seen global adoption of constructed wetlands and a significant increase in its implementation by many countries, industries, municipalities and communities. Research and technological innovations are currently being carried out to improve the designs of constructed wetland systems, improve the capacity to remove nutrients, and modify the technology to operate in various climates and wastewater concentrations. The utilization of different natural and advanced materials and monitoring systems has been explored in various wetland designs.

Nowadays, constructed wetlands are essential components of integrated water treatment system. In addition to treating wastewater, they are used for biodiversity preservation, storm water management, and climate change adaption. The use of constructed wetlands as a natural approach to water treatment and environmental conservation has acquired worldwide recognition. Initiatives and global organizations support their implementation to achieve sustainable development goals. Future developments in constructed wetland technology will probably continue to involve increased scalability, better integration with other water management systems, and innovations for performance enhancement. Constructed wetlands have evolved historically, from early trials to mainstream recognition and acceptance as an important sustainable water management technology. Ongoing research will continue to shape their role in addressing global water scarcity issues.

2.3. Development of constructed wetland technology in India

In the early nineties there were thousands of constructed wetlands worldwide that were used to treat wastewater from municipal, industrial sources, agricultural, urban and strome runoff. Such systems received good popularity in Northern Europe and USA. Around this time, in spite of the abundant information available in the west about such systems, negligible efforts were made to explore utilization of these artificial systems in developing countries like India which lack proper sewage collection and treatment facilities (Billore et al., 1999).

In India, the utilization of plants and microbes based treatment system was relatively unexplored until early 2000s. The very first study using constructed wetland to treat wastewater was reported by Juwarkar et al in the year 1995. Around this time several hundred wetlands were already operational in many countries like Austria, Denmark, Italy, Brazil, and more. The very first system in India was installed in Bhubaneshwar for the removal of organic contaminants from primary treated domestic wastewater. Constructed wetland was filled with 70% sand and 30% soil and *Typha latiforlia* and *Phragmites Carca* were planted in it. Four weeks after initial plantation, primary treated wastewater was entered into the wetland unit following a downflow system. It was reported to be efficient in the removal of BOD (67-90%) and N (58-63%). Treated effluent was proposed to be utilized for fish pond, forestry and horticulture. The study recommended the use of this cost-efficient alternative for adoption in small towns and villages (Juwarkar et al., 1995).

In 1999 Billore et al used indigenous tropical emergent grass species, *Phragmites Karka* planted in gravel bed for treatment of municipal wastewater. Field scale horizontal subsurface flow CW was used to remove organic contaminants from domestic wastewater coming from staff quarters on Vikram University campus in Ujjain, Madhya Pradesh. Based on the available land area the system was constructed and no specific design was followed for sizing the wetland in the study. The system was designed to have an earthen channel pre-treatment unit and a horizontal subsurface CW. Wastewater added into the wetland unit was first pre-treated by passing it through a wide cement pipe filled with graded boulders of 4-11 cm followed by a shallow open ditch having *Echinichloea colonum*, a perennial grass grown in it. Pre-treated water was then entered into the main SF root zone treatment which was found to remove 78% NH_4-N and TSS and 58-65% P, BOD and TKN. The system was also found to enhance 34% dissolved oxygen saturation in the effluent. The work highlighted the suitability of such systems in tropical country with suitable climatic conditions for rapid plant growth (Billore et al., 1999).

Another work was reported by the same group in the year 2001 for removal of organic contaminants from molasses-based industrial distillery effluent. The system was having a pre-treatment chamber followed by 4 serial celled HSSFCW treatment cells (C1 to C4). Effluent after secondary treatment was made to enter into an open three-partitioned pre-treatment chamber containing round gravel (8–12 cm) in the first chamber, a baffle of a half partition wall in the second chamber which allowed only the supernatant to be passed into the third chamber. Wastewater was transferred from pre-treatment unit to series of gravel beds C1- C2- C3 and C4. Gravel and plants used in the study were collected locally. Unplanted C1 gravel bed was for anaerobic treatment. An addition aeration system was provided in the medium voids filled with influent in unplanted C2 having round gravel of size 2–5 cm. Porous baked earthen bowls were installed in this unit in an inverted position, horizontally in three tiers to have air pockets in the bowls. C3 gravel bed planted with *Typha latifolia* was having a wide strip of brick rubble band inserted in it to enhance phosphorus removal. C4 was planted with *Phragmites karka*. Total 14.4 days of retention was provided in the four wetland units. The system was found to be efficient in removal of BOD (80-90%), COD (60-70%), TKN (55-60%), NH_4-N (50%), NO_3-N (60%), TP (80%), TS (40%), TSS (50%), and TDS (40%). Significant reduction in contaminants was observed and the potential of CW technology over the poorly performing conventional treatment systems were highlighted (Billore et al., 2001)

In India, constructed wetlands have been used to treat wastewater since the late 20th century. Most of the early initiatives investigated the viability of treating wastewater with natural systems, usually on a small experimental scale. The advantages of constructed wetlands for the environment and the economy were becoming more widely acknowledged in the early 2000s. To evaluate the effectiveness of these systems in treating various wastewater types in both rural and urban settings, pilot studies were undertaken. Increased scale-up of constructed wetlands was witnessed in many Indian states throughout the late 2000s and early 2010s. These solutions were embraced by businesses, municipalities, and environmental organizations as components of their wastewater management plans.

Research and invention concerning constructed wetlands experienced a significant upsurge during the 2010s. Around this time research mostly centred on maximizing design parameters, comprehending ecological factors, and modifying these systems to accommodate a range of environmental conditions. Applications for constructed wetlands were found in many different fields, such as decentralized solutions for rural areas, industrial effluent treatment, and urban wastewater treatment. These systems' adaptability and diverse applications were becoming increasingly evident. Around 2019 Indian government (DBT and CPCB) introduced rules and regulations to promote the implementation of constructed wetlands (DBT-CBCP Manual, 2019). These recommendations provided a framework for the design and management of constructed wetlands for wastewater treatment. Constructed wetlands are a crucial component of India's sustainable wastewater management strategies as of this decade. The research on constructed wetlands continues to expand due to ongoing government assistance, and the increased emphasis on environmental conservation and biodiversity enhancement.

Currently, various constructed wetland-based treatment technologies are operational across the globe. One such technology is the "Phytorid Technology," which is a scientific wetland with active biodegradation (SWAB) developed and patented by CSIR-National Environmental Engineering Research Institute (NEERI) for wastewater treatment. The first-ever Phytorid system was installed in the year 2006 on the Kalian campus of Mumbai University. Presently, more than 35 such systems are operational in different parts of India (CSIR-NEERI, 2020). Additionally, the Indian Agricultural Research Institute (IARI), Pusa, New Delhi, has a constructed wetland on its campus since 2012, having a capacity of 2.2 MLD and an annual irrigation potential of 132 Ha. The developed wetland treatment system was found capable of removing turbidity (99%), BOD (87%), nitrate (95%), phosphate (90%), and heavy metals (81–99%) according to long-term monitoring of its treatment performance (IARI, 2014). Several cooperation projects like “Safeguarding Water resources in India with Green and Sustainable technologies (SWINGS)” between the European Union and India are also attempting to develop and implement low-cost wastewater management technologies with constructed wetlands (CW) as the primary unit integrated with a high-rate anaerobic system and solar-driven disinfection technologies. Under this project, pilot plants were operational from 2012 to 2016 and were fully commissioned in 2016 in Aligarh Muslim University, AMU (Aligarh, Uttar Pradesh, Northern India). The International Centre for Ecological Engineering of the University of Kalyani (Kalyani, West Bengal, Eastern India) and the Indira Gandhi National Tribal University (IGNTU) (Lalpur and Amarkantak, Madhya Pradesh, Central India) also developed constructed wetland pilot plants under SWINGS project (Álvarez et al. 2017).

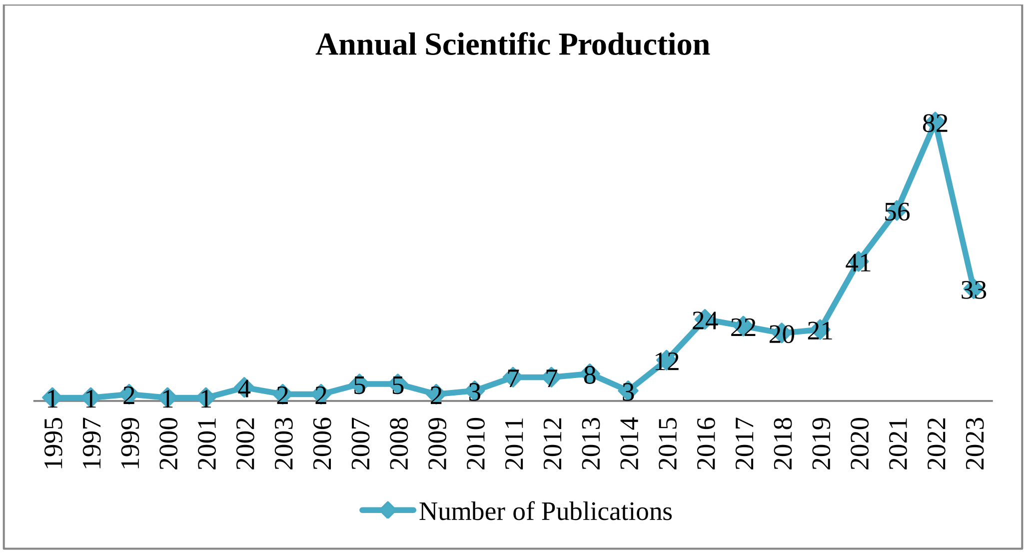


Fig. 2.1 Increase in constructed wetland studies in India over the years

Over the years considerable increase in the publications on constructed wetland studies have been observed (Fig. 2.1).The rise in constructed wetland research in India after 2015 can be attributed to growing concerns about water quality, increased awareness of environmental sustainability and the necessity for affordable wastewater management techniques. Moreover, government initiatives and increased awareness among the masses regarding environmental concerns like water scarcity and deterioration of existing water bodies, encouraged research in this area. Furthermore, the adoption of constructed wetlands was also in alignment with India's emphasis on economical and environmentally sound ways to mitigate water pollution. By offering habitats to a variety of plants and animals, these systems support the overall ecological balance as well. Constructed wetlands have been acknowledged as important by government and environmental organizations. Establishing these systems as a component of sustainable water management practices is being encouraged by policies and initiatives that have been created. Constructed wetland can be a decentralized and economical alternative to traditional wastewater treatment plants in rural areas, and they also offer an alternative technology in urban areas. Increasing awareness among the public about the advantages of constructed wetlands can contribute to its growth. To educate communities about the benefits of these natural treatment systems, outreach initiatives, and educational programmes can be conducted. Further, the long-term sustainability of these ecologically beneficial approaches can be ensured by community involvement, which promotes a sense of ownership. Constructed wetland development has grown as a sustainable wastewater treatment option in India due to government assistance, research endeavours, stakeholder engagement, and environmental awareness.

2.4. Constructed wetland components

Three main components of the engineered wetland systems are porous-filter media, vegetation and microorganisms (Fig. 2.2). Materials like sand, soil and gravels are used to create permeable filter media or substrate which supports plant growth. In addition to this it also provides ample space for the microbes to attach, flourish and forms biofilm structures (Sandoval et al., 2019a). Vegetation provides root zone area also called as rhizosphere which contains air channels that transport oxygen into the filter bed. Most of this oxygen is used to carry out respiration but some of it is lost to the surroundings which supports aerobic degradation of organic matter by hetrotrophic, ammonifying and nitrifying bacteria. Regions where limited or no oxygen supply is available, anaerobic respiration by either facultative or obligate anaerobes like sulfate reducing and methane-forming bacteria play an important role (Vymazal, 2005).

Various types of materials ranging from industrial by-products to natural and artificial sources have been used as substrates in different studies. Mostly soil, gravel and sand are used as substrates in CWs. Substrates provide the main physical structure of the system for processes like settling, adsorption, and retention of pollutants. Substrates play an important role in the removal of contaminants via various processes like filtration, adsorption, precipitation, etc. The substrate is an essential component that determines hydraulic performance, supports plant growth, microbial adhesion, and diversity thereby influencing the overall efficiency of the wetland system (Shen et al., 2020).

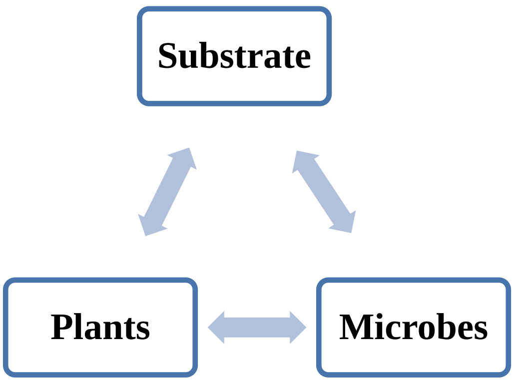


Fig. 2.2 Components of constructed wetlands

Presence of plants in the system not only enhances the wildlife habitat value and aesthetic appearance but stabilizes the sediments, supports the physical structure, and enhances the transformation of contaminants in the rhizosphere (Stottmeister et al., 2003). In addition to reducing the current velocity, plants mainly provide surface area for microbial growth and activity. Plant roots also provide oxygen in the rhizosphere. Plants also insulate the surface from frost (Brisson and Chazarenc, 2009).

Tanner (2001) reported that planted systems provide little improvement in suspended solids, BOD, and COD removal but enhances the removal of nitrogen and phosphorus from wastewater. The quantity of nutrients accumulated in plant biomass varies among the plant species. Generally, a small proportion of the nutrients are removed by direct plant uptake. Plants mostly facilitate the process by sequestration of accumulated organic matter and enhancing nutrient transformation. Planted systems have high organic matter accumulation on the surface over years due to the decomposition of plant litter which provides additional sorption sites and is also a source of biochemically active substances which enhance microbial processes like nitrification and denitrification (Stottmeister et al., 2003).

With time diverse microbial populations such as bacteria, fungi, protozoa, algae, and yeasts develop in the system. These microbes play a key role in the transformation and mineralization of pollutants. Microbes metabolize nutrients and organic pollutants through various processes like nitrification, denitrification, sulfate reduction, and methanogenesis, etc (Stottmeister et al., 2003). Both aerobic and anaerobic bacterial conversions and natural decay are significant processes in the transformation of toxic organic compounds (Peterson, 1998). Most of the initial constructed wetland studies were focused on influent and effluent physiochemical assessment with little attempts to explore the microbial populations prevalent in the system. In the last few years, researchers have started characterizing microbial populations as it provides important information about understanding their role in the treatment process. Few studies have reported the presence of *Nitrosomonas, Nitrosospira, Proteobacteria, Actinobacteria, Firmicutes, Chloroflexi, Bacteroidetes* species in the plant roots zone. The presence of methanogens and sulfur degrading bacteria is also reported in the literature (Bharagava, 2020). Recently, metagenomic analysis of the soil samples is also being increasingly employed to analyze the microbial communities present in the wetland system. Characterization of microorganisms and the range of bacterial genera might provide valuable information about the removal of organic compounds, and nutrients in the system (Rampuria et al., 2021).

2.5. Treatment processes taking place in a wetland system

Various processes in CWs that improve water quality include physical, chemical, and biological removal of contaminants (Fig. 2.3). It involves the settling of suspended particles, filtration, adsorption, chemical transformation, ion exchange on the plant surface, uptake of nutrients by plants, and breakdown of pollutants by microbes (Tanaka et al., 2011). Transformation processes like oxidation, reduction, and biogeochemical conversions also take place in CWs (Kumar and Dutta, 2019). The root zone also known as the rhizosphere is the active reaction zone in the system where all components of the system i.e. plants, microbes, and pollutants interact with each other. Most of the biological degradation and physiochemical processes take place in this region as a result of these interactions. Root zone oxygen release has an important role in the aerobic degradation of pollutants, in nutrient transformation and sequestration (Stottmeister et al., 2003).

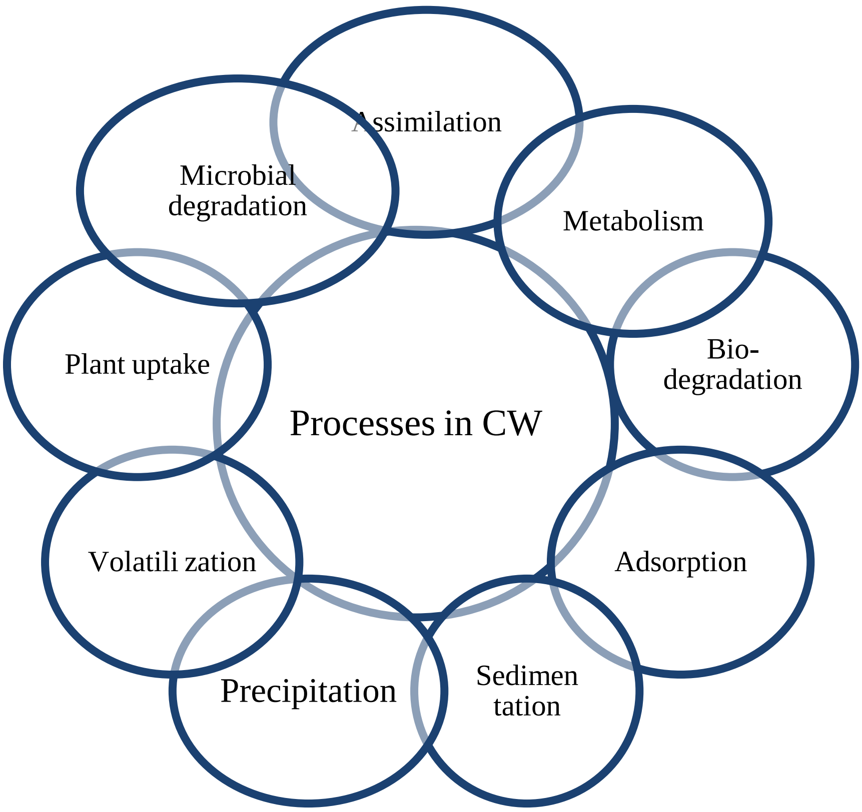


Fig.2.3 Treatment processes taking place in constructed wetlands

2.6. Selection of suitable plant for the wetland system

Although the positive and significant role of plants in removal of pollutants is well established and already demonstrated by various studies comparing planted and unplanted systems, but choosing the appropriate plants for the system remains a challenge. There is little generalization that could guide in the selection of appropriate species of plants for constructed wetland system (Brisson and Chazarenc, 2009).There is no proper guideline that could help in selection of suitable plants and substrates for the constructed wetland (Parde et al., 2021). More often than not selection of plants was based on the established practices rather than the rigorous comparative assessment of the efficiencies of various plants in wetland conditions. Majority of the constructed wetland studies were performed with few widely used plants like *Phragmites*, *Typha* and *Canna*. Relatively few studies have explored the utilization of ornamental plants that have commercial value and can survive wastewater stress in wetland systems (Sharma et al., 2023). Using constructed wetlands for growing ornamental plants is an added advantage that can boost the adoption of these systems. Some commonly used plants in the constructed wetland systems around the globe are presented in Fig.2.4

Emergent Plants

- *Phragmites australis, Scirpus, Typha, Sagittaria latifolia, Bolboschoenus, Fluviatilis, Eleocharis sphacelata, Iris, Juncus*

Submerged Plants

- *Hydrochairs morsusranae, Myriophyllum speculum, Elodea canadensis, Potamogeton pectinatus, Vallisneria americana, Hydrilla verticillata, Ceratophyllum demersum*

Floating Plants

- *Eichhornia crassipes, Pistia stratiotes, Duckweed, Lemna minor, Spirodela, Polyrhiza, Wolffia, Hydrochairs dubia*

Ornamental Plants

- *Canna, Iris, Heliconia, Zantedeschia, Hedychium coronarium, Tulbaghia violácea, Alpinia purpurata Heliconia, Gladiolus, Strelitzia reginae*

Fig. 2.4 Plants used in constructed wetlands

Phragmites, Typha, Scirpus, and *Juncus* plant species are the most documented ones in constructed wetlands around the globe for many decades. Comparatively, the number of ornamental plant species evaluated in these systems is very low (Burgos et al., 2017). The use of ornamental plants in the constructed wetlands is an attractive option not only owning to its aesthetic and commercial value but also because of the other added benefits that include ecosystem services and biodiversity enhancement. Experiments in the recent past have demonstrated the ability of different ornamental plants in the wetland system (Calheiros et al., 2015). Constructed wetlands with ornamental plants have been reported to be efficient in the removal of BOD, COD, ammonium, and phosphate. Recent studies have reported high removal (above 80%-90%) of pollutants like organics, coliforms, and nutrients using constructed wetlands with different ornamental plants (Calheiros et al., 2015; Marín-Muñiz et al., 2020). Similarities have been observed in the performance of constructed wetlands using common plants and ornamental plants. Accordingly, the use of different species of ornamental plants has increased over the years (Sandoval et al., 2019b). However, the influence of various commercially valuable ornamental plants is not much evaluated and compared (Burgos et al., 2017). Marín-Muñiz et al. (2020) highlighted the need for a better understanding of the functionality of ornamental plants in constructed wetlands. Using ornamental plants in the wetland system add a new dimension to its adoption and aesthetic appearance (Haritash et al., 2015)

2.7. Selection of suitable substrate for the wetland system

The selection of an appropriate substrate and plants in the design of a constructed wetland system to treat wastewater is the most important design parameter that can significantly enhance the efficiency of the system (Ji Z et al., 2022). Selection of appropriate substrate improves operation cycle, enhances pollutant removal and avoids clogging (Wang Y et al., 2020). Various substrates used in the constructed wetland systems around the globe are presented in Fig.2.5

Natural materials

- Sand, Gravel, Clay, Calcite, Marble, Vermiculite, Limestone, Shell, Shale, Peat, Zeolite,Organic wood mulch

Artificial products

- Activated carbon, Light weight aggregates, Compost, Calcium silicate hydrate, Ceramsite, Basic oxygen furnace slag (BOFS), Light ceramsite

Industrial byproducts

- Slag, Fly ash, Coal cinder, Alum sludge, Hollow brick crumbs, Moleanos limestone, Wollastonite tailings, oil palm shell

Agricultural byproducts

- Rice husk, Rice staw, Sugarcane bagasse, Coconut husk, Wood chips, Corn stalks, Peat moss, Composted organic materials, Biochar, Sawdust or Wood Shavings, Coconut Coir, Wheat Straw

Fig.2.5 Substrates used in constructed wetlands

Constructed wetlands are extremely versatile systems in terms of their design, kind of media used, variety of plant species grown and the type of wastewater being treated. Many studies have highlighted the potential of using a wide variety of substrates and ornamental plants in constructed wetlands (Sandoval-Herazo et al., 2018; Kumar and Dutta, 2019). Different types of wastes like industrial waste, plastic waste, and other artificial materials have been used as substrates in the wetland system, in an attempt to reduce the cost, but comparatively little work has been done in exploring the use of biological materials as wetland substrates.

Utilizing agricultural waste as substrate in constructed wetlands promotes the sustainable use of organic materials while enhancing the treatment capacity of the system. Few recent studies have reported that agricultural residues and biochar produced from them can be used as additives or substitutes for conventional growing media (Ajibade et al., 2021). Incorporation of these organic materials will improve the filter bed properties of the system mainly by increasing the carbon source and improving the surface adsorption of pollutants on the media. Constructed wetlands also offer a great possibility for adaptation to local constraints (Calheiros et al., 2015).

In fact, building these systems using locally available media components and native plants will them more cost-efficient as well as better suited to the conditions and requirements of a specific region.

2.7.1. Impact of intensive agricultural practices on natural resources

India being an agrarian economy is a chief producer of agricultural products. Food grains like rice, wheat and pulses form main part of the stable diet of major fraction of the population. In the year 2016-17, 110.15 million tonnes of rice, 98.38 million tonnes of wheat, 44.19 million tonnes of coarse cereals and 22.95 million tonnes of pulses were produced (Krishi Annual Report, 2017-18). These crops generate huge amount of agricultural waste, cereal crops like rice and wheat have major contribution in generation of crop residues (NPMCR, 2014). Punjab is a major contributor of wheat and rice to the central pool. Majority of the food grains grown in the region goes into public distribution system. Agriculture in most of the northern states and especially in Punjab is virtually a rice-wheat monoculture (Kumar et al., 2015). Intensive agricultural practices in the region have put considerable strain on soil and water natural resources. Overexploitation of the groundwater sources for irrigation purposes is another major problem in the region.

2.7.2. Management of crop residues

As per Indian Ministry of New and Renewable Energy (MNRE) 500 million tons (Mt) of crops reside is generated per year. These crops residues are presently used as fodder, fuel and for other domestic and industrial purposes. In spite of its various uses still a significant part of these residues are burnt on field. Rotation of rice and wheat crops leaves a small window of days to manage the crop residues. Because of lack of suitable agri-waste management practices and short time, labor, handling cost involved in removing crop residues, a large portion of it is burnt on the fields (NPMCR, 2014). There is surplus of 140 metric tons out of which nearly 92 metric tons of these residues are burnt every year (Bhuvaneshwari et al., 2019).

Currently, the burning of agricultural residues left on the fields after harvesting is a common practice in many parts of the world. Burning of these residues releases large quantities of greenhouse gases and particulate matter into the environment affecting air quality and climate change. The Food and Agriculture Organization reported 21% of greenhouse gas emissions are through the agriculture sector (FAO, 2017). Crop residue burning generates green house gases and other particulate matter comprising aerosols and hydrocarbons. Carbon present in the crops is emitted as CO_2, CO and CH_4 which can cause serious environmental problems. Particulate matter release in the air from burning can deteriorate air quality and leads of various air borne health problems. Burning of crops also results in loss of essential nutrients like nitrogen, phosphorus, sulphur and potassium which could have been used to enrich soil. Burning also elevates temperature of the top soil which destroys the beneficial soil microbial community (NPMCR, 2014).

These residues have economic value as bio manure, compost making, cattle feed, fuel for domestic cooking, mulching material, power generation, biofuel, energy sources, etc. Post-harvest residues are increasingly being employed in all these alternative applications but still, nearly 234 million tonnes per year of residues are surplus (Devi et al., 2017; Lohan et al., 2018). The use of agricultural waste as components of constructed wetland substrate is gaining popularity due to its low cost, easy availability, effective pollutant removal ability, and minimized waste disposal (Yang et al., 2018).

2.7.3. Utilizing agricultural waste as substrates in constructed wetlands

India is a largely agricultural country and is facing the challenge of the disposal of huge quantities of crop residues. More than 600 million tonnes of organic waste are generated annually in India from various agricultural activities like crop production, harvest, and agro-industrial processing (Pappu et al., 2007; Prusty et al., 2016). It includes approximately 122 million tonnes of rice and 141 million tonnes of sugarcane produced annually. Rice straw and sugarcane bagasse are abundantly available agricultural byproducts in many Indian states. Nearly 371 million tons of crop residues are generated annually in India. 27% –36% of which are wheat residues and 51% – 57% are paddy residues.

These residues contain plant nutrients that can be utilized as part of fertilizers or manures. Addition of residues also improves soil structure, fertility and microbial communities present in the soil (Lohan et al., 2018). Crop residues have been traditionally used for preparing compost. These residues can be used to improve soil properties in various forms like particles, ash, and biochar. Biochar is a high carbon material produced through slow pyrolysis of the biomass. Crop residue application helps to enhance organic matter cover and promotes soil biological activity. Incorporation of crop residues into soil reduces bulk density of soil and increases hydraulic conductivity and cation exchange capacity by modifying soil structure. Management of crop residues with conservation agriculture is vital for the long-term sustainability of Indian agriculture (Gupta and Dadlani, 2012).

Some abundantly available agricultural wastes than can be utilized as substrates in constructed wetlands are rice husk as their high porosity facilitates good aeration and water retention; bagasse due to its high carbon content and porosity; sawdust and wood chips that offers large surface area to support microbial activity and the formation of biofilm; coconut coir as it can support microbial population, is lightweight and has an excellent capacity to hold water. Other agricultural wastes that can be explored as wetland substrates include corn cobs, palm kernel shells, **s**traw and hay from crops etc. Utilizing organic waste as substrate in constructed wetland treatment systems will help to address the problem of agricultural waste disposal as well.

2.8. Bibliometric analysis of plants and substrates used in the system

Bibliometric analysis have been used previously to trace the developmental footprints of diverse aspects of constructed wetland technology, for instance, it has been used to review the research development in CWs over the years, identify phosphorous removal pathways and uncover the current scenario and key themes of constructed wetlands research (Zhi and Ji, 2012; Dell'Osbel et al., 2020; Ji B et al., 2021). Bibliometric studies that focused on substrates and flowering plants used in CWs are listed in Table 2.1

Table 2.1 Bibliometric studies focusing on constructed wetland research

Title	**Years considered**	**No of Publications**	**Source Database**	**Tools used**	**Keywords used**	**Extracted from**
"Application of ornamental plants in constructed wetlands for wastewater treatment: A scientometric analysis"	2002–2022	114	Scopus	VOS viewer	"ornamental plant" AND "constructed wetlands"	title, abstract and words key
"Bibliometric Analysis of Constructed Wetlands with Ornamental Flowering Plants: The Importance of Green Technology"	2000–2022	10,254	DIMENSIONS	DIMENSIONS program	"constructed wetland", "artificial wetland", "treatment wetland", "wetland biofilter", "engineering wetland", "ecotechnology wetland"	title and abstracts
"Bibliometric Analysis of Phosphorous Removal Through Constructed Wetlands"	1995-2019	2020	Web of Science	VOS viewer	"Phosphorus removal in Constructed Wetlands"	Title and Abstracts
"Constructed wetlands, 1991–2011: A review of research development, current trends, and future directions"	1991-2011	2883	SCI-EXPANDED		"constructed wetland", "constructed wetlands", "engineered wetland", "engineered wetlands", "artificial wetland", "artificial wetlands", "treatment wetland", "treatment wetlands", "reed bed", and "reed beds"	title search

In the present study, a bibliometric analysis of 364 publications extracted from SCOPUS database was done to (1) identify the wide range of substrates utilized in the constructed wetlands and highlight the substrates that have been reported as viable alternatives to the conventional media used in the constructed wetlands i.e. soil, sand and gravels, (2) determine various ornamental plants that have been investigated in wetland systems instead of the commonly preferred wetland plants like *Phragmites, Typha* and *Canna*, (3) identify the contribution of various countries, authors and collaborations in constructed wetland research.

A total of 331 publications were found having the keywords “constructed wetland” AND “substrate” in the title whereas only 33 publications had “constructed wetland” AND “ornamental plant” in the title. The article reviews 30 years of study on the use of artificial wetlands for the treatment of water and wastewater. Both CSV files were merged resulting in a total of 364 publications analyzed to explore the wide range of substrates and ornamental plants used in these wastewater treatment systems. These studies were carried out in diverse climatic conditions utilising a variety of substrates and plant species in varied experimental setups to treat different types of effluents.

The annual scientific publications on constructed wetlands showing effect of substrate and plants over the previous 20 years is illustrated in Fig. 2.6. The data shows a consistent increase in research over time, with two notable peaks in the number of publications: in 2011 there were 23 publications, up from 9 in 2010; in 2021 there were 29, and in 2022 there were 43 publications. This emerging trend indicates that although research in this field has been done for a long time, the scientific community has only recently begun to pay it serious consideration.

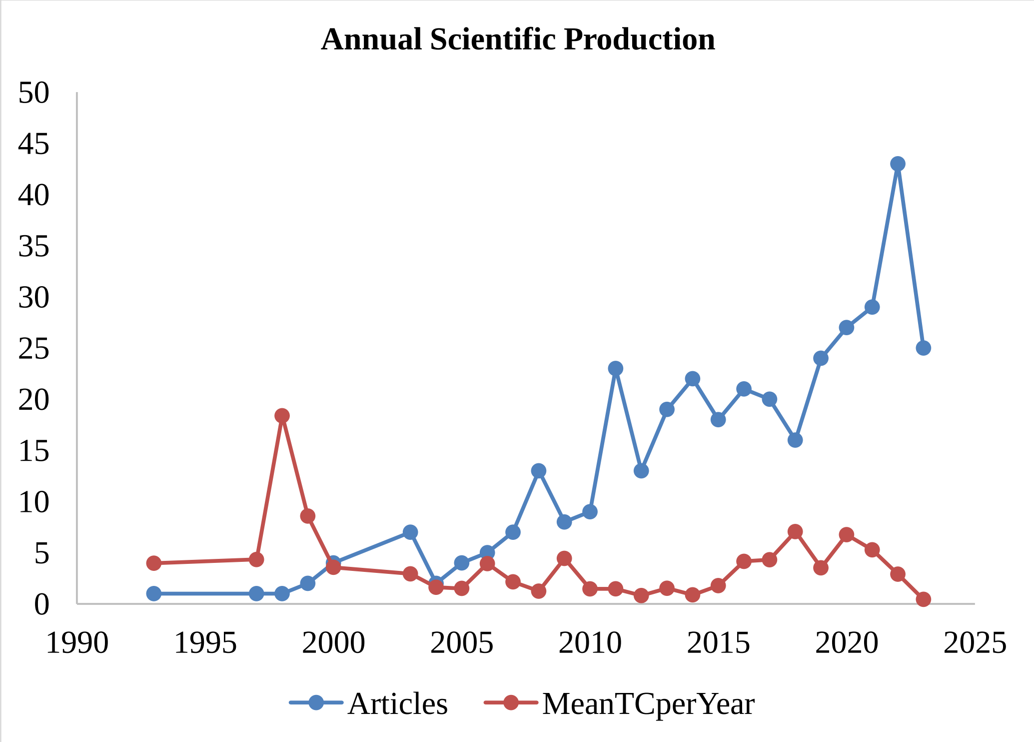


Fig. 2.6 Graph depicting the annual scientific production and the mean citation of publications per year

The initial attempt of treating wastewater using wetland plants was done by Dr. Kathe Seidel in the early 1960s at Max Planck Institute, Germany. However the work exploring other substrates was first reported in 1993 by Mann R.A and Bavor H.J. As per the mean citation depicted in Fig. 2.6, the most cited paper was on "The Phosphate adsorption characteristics of soils, slags and zeolite to be used as substrates in constructed wetland systems" by Sakadevan K. and Bavor H.J. published in 1998 setting the initial base as well as the pioneer research in this area having a mean of 18.38 citations per year. Using appropriate substrates, the efficacy of artificial wetlands at removing pollutants from wastewater was assessed. In order to determine possible substrates for phosphorus removal in artificial wetlands, the phosphorus (P) adsorption capabilities of soils, two industrial by-products, and a clinoptilolite material (zeolite) were studied in this work. Followed by the paper published in the year 1999 by Drizo et al. on "Physico-chemical screening of phosphate-removing substrates for use in constructed wetland systems" having mean citation of 8.58 per year and in 2018 by Yang et al. on “global development of various emerged substrates utilized in constructed wetlands” having a mean citation of 7.06 per year.

The increase in the publication over year can be sought to increased interest of researchers towards this particular area. Constructed wetlands were given priority in the search for effective and affordable wastewater treatment methods because of the numerous benefits, including reduced expenses in comparison to alternative treatment methods, a high level of resilience to variations in water flow that are typical in cities, the creation of an environment which is favourable for wetland organisms, and harmonious integration with the landscape around it.

Data was further reviewed to identify most relevant authors (Table 2.2) and most cited authors (Table 2.3) that contributed in constructed wetland research. Most relevant sources are listed in Table 2.4 and countries that collaborated in various research projects are listed in Table 2.5. Bibliometric analysis show that between 1993 and 2023, the growth of CW research was expanding exponentially. Scientists from different nations contributed to this enormous body of research and study, with China taking the lead followed by Mexico, Ireland, Brazil and USA. WANG H has the highest number of publications and LIU Y is the most cited author.

Table 2.2 Top ten relevant authors

Authors	Number of Articles	Articles Fractionalized
WANG H	18	3.50
XU D	16	2.95
ZHANG J	16	2.38
LI X	15	2.54
HE F	14	2.71
LI Y	12	2.23
YANG Y	12	2.04
XU J	11	2.50
ZHAO YQ	9	2.76
BABATUNDE AO	7	2.25

Table 2.3 Top ten cited authors

Author	Local Citations
LIU Y	102
LI X	81
WANG S	58
ZHANG L	50
HE F	49
LI M	46
SONG X	43
WU Z	40
WANG W	39
CALLIER MD	38
LIANG Z	38
MA X	38
ROQUE D'ORBCASTEL E	38
SUN G	38

Table 2.4 Top ten most relevant sources

Sources	Articles
ECOLOGICAL ENGINEERING	27
ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH	23
WATER SCIENCE AND TECHNOLOGY	22
BIORESOURCE TECHNOLOGY	17
CHINESE JOURNAL OF ENVIRONMENTAL ENGINEERING	15
SCIENCE OF THE TOTAL ENVIRONMENT	14
HUANJING KEXUE/ENVIRONMENTAL SCIENCE	12
JOURNAL OF ENVIRONMENTAL MANAGEMENT	10
WATER RESEARCH	10
ZHONGGUO HUANJING KEXUE/CHINA ENVIRONMENTAL SCIENCE	10

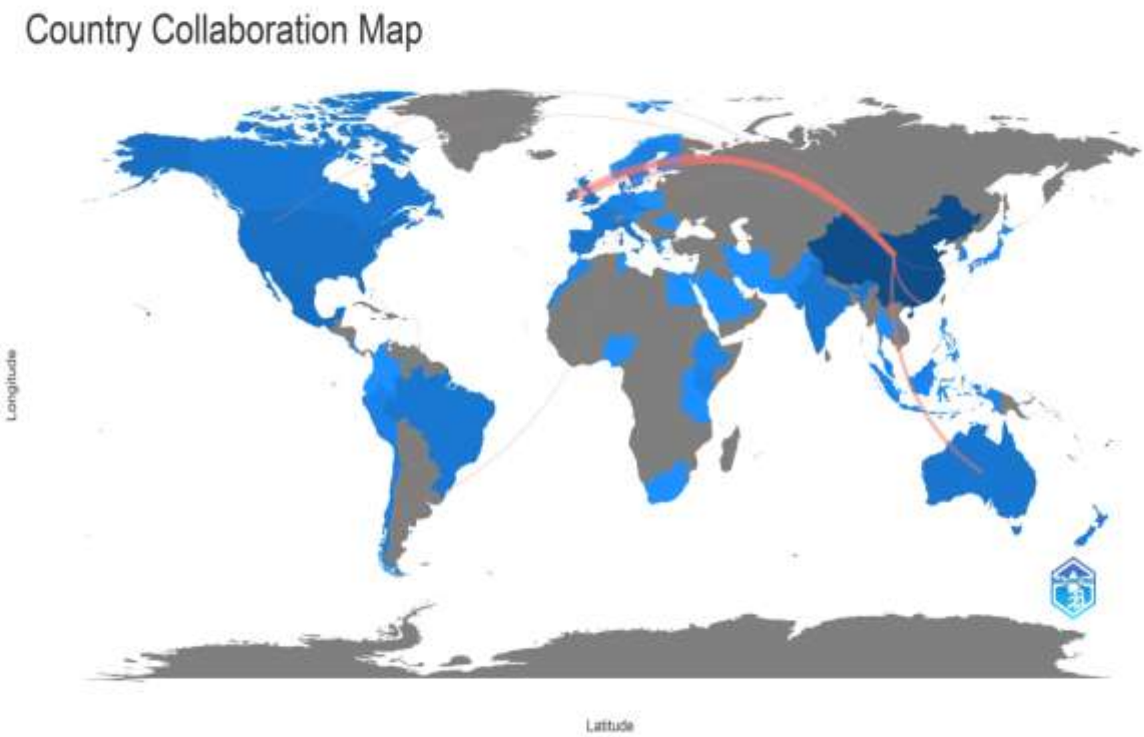


Fig 2.7 Collaboration of countries working in the area of constructed wetland (Created by Biblioshiny)

Table 2.5 Countries that are collaborating

From	To	Frequency
CHINA	IRELAND	7
CHINA	UNITED KINGDOM	7
CHINA	AUSTRALIA	4
CHINA	DENMARK	3
CHINA	HONG KONG	3
IRELAND	UNITED KINGDOM	3
CHILE	DENMARK	2
CHINA	CANADA	2
CHINA	CZECH REPUBLIC	2
CHINA	FINLAND	2

Choice of an appropriate substrate is a very important criterion that influences nutrient removal. Applications of different natural and artificial materials have been reported to improve phosphorous removal. Thus, selection of a suitable substrate material is one of the main aspects of the design and performance output (Dell'Osbel et al., 2020). Various plants and substrates used in constructed wetland studies and their influence on the pollutant removal efficiency is presented in table 2.6.

Table 2.6 Studies that used ornamental plants and different substrates in constructed wetlands along with their pollutant removal efficiency

References	**Location Country**	**Type of Constructed wetland**	**Type of waste water (WW)**	**Number of plants**	**Plants used (Scientific name)**	**Substrate No**	**Substrates used (Names)**	**HRT (days)**	**Removal Efficiency of pollutants (%)**	**Effect of Plant**	**Effect of Substrate**
2023- Stefanatou et al.	Mytilene, Greece	VSSF CW	Greywater	3	*Trachelospermum jasminoides, Lonicera japonica, Callistemon laevis*	2	Sand, vermiculite	1	COD: 73-96, Turbidity:54-94	Positive	Positive
2023- Liu et al.	Beijing, China	VSSF CW	Synthetic WW	1	*Iris pseudacorus*	4	Iron-carbon (Fe-C), Pebble, gravel, quartz sand	3-4	COD:84.4, NH_4^+-N:94.0, NO_3^--N:81.1, TN:86.6, TP:84.3	Positive	Positive
2022- de la Cruz Magaña et al.	UnB - Brasilia, Brazil	VSSF CW	Raw domestic WW	3	*Cyperus giganteus, Typha* sp., *Eichhornia crassipes*	1	Soil (red-yellow latosol, oxisol or gibbsic)	5.09 -6.6	P:34.33, K+:37.88, Ca2+:39.82, Fe:45.4	No effect	Positive
2022- Qingwei et al.	Hangzhou, China	Parallel subsurface flow CW	Domestic sewage	1	*Pontederia* sp.	1	Magnetic Fe_3O_4 nanoparticles (F_e3O_4 NPs)	6	NH_4^+-N:95.9, NO_3^--N:83.8, TP:90.4	No effect	Positive
2021- Aregu et al.	Modjo, Ehiopia	HSSF CW	Tannery WW	1	*Chrysopogon zizanioides*	1	Pumice substrate	7and 9	BOD_5: 96.30, COD: 96.91, NO_3-N: 99.68, TN: 99.00, PO_4-P: 100, TP: 96.17, Chromium: 98.91	Positive	Positive
2020- Rahmadyanti and Wiyono	Indonesia	VSSF CW	Batik WW	1	*Canna indica*	1	Rice husk	21	TSS: 91.25, BOD_5: 91.82, COD: 89.15, ammonia: 96.2, heavy metals (Cr:	Positive	Positive

									81.8)		
2020-Yuan et al.	Jinan,China	VSSF CW	Synthetic WW	1	*Acorus calamus*	2	Alum sludge, wood chips	3	NH_4^+-N 19.0–75.3, NO_3^--N: 63.6–96.1, TN: 61.94–74.4, TP: 75.0–98.8, C/N ratio: 0.93-1.87	Positive	Positive
2019-Zamora-Castro et al. 2019	Pastorías, Mexico	Fill-and-drain CW	Rural community WW	3	*Canna indica, Pontederia sagittata, Spathiphyllum wallisii*	2	Porous river rock, Tepezyl	3	COD:81-83, BOD_5:80-84, TKN:61-69, NO_3^-N:61-68, NH_4^+-N:65-71, $PO_4^{3—}$P:62-68	Positive	Positive
2019- Das et al.	Tamil Nadu, India	Integrated MFC CW	Domestic WW	1	*Canna indica*	2	Gravel	21-25	COD: 83.1-88.1, TDS: 77.5-82.05	Positive	Positive
2018-Hernández et al.	Veracruz, Mexico	SFCW and SSFCW at high and low density	Municipal WW	3	*Zantedeschia aethiopica, Typha* sp., *Cyperus papyrus*	2	Upland soil, volcanic gravel	3	CH_4 emission: 436-518 $m^{-2}.d^{-1}$, N_2O emission: 17-23 $m^{-2}.d^{-1}$ N-NH_4:0-96, N-NO_3:12-55, P-PO_4:26-62, TOC:31-53	No effect	Positive
2018-Sandoval-Herazo et al.	Misantla, Mexico	VSSF CW	Domestic WW	3	*Lavandula* sp.*, Spathiphyllum wallisii, and Zantedeschia aethiopica*	2	Red volcanic gravel (RVG), Polyethylene terephthalate (PET)	5	PO_4^{3-}-P: 35-38 , n NO_3^--N: 35-50, BOD_5:63-68, fecal coliforms:59-64	Positive	Positive
2017-Bakhshoodeh et al.	Isfahan, Iran	HSSF CW	Municipal solid waste	1	*Vetiveria zizanioides*	1	Compost	5	BOD_5: 75%, COD: 53%, NH_3-N: 70, NO_3-N: 74, TN: 74	Positive	Positive
2017-Jin et al.	Kingston, Canada	surface	Municipal	1	*Typha latifolia*	1	Peat	2.5	NO_3^--N: 92.7, TN:75.0	Positive	Positive

		FCW	WW								
2016-Chen et al.	Guangzhou, China	Mesocosm-scale horizontal SSFCW	Domestic WW	1	*Cyperus alternifolius*	4	Oyster shell, zeolite, medical stone and ceramic)	1	Antibiotics: 17.9-98.5, Total antibiotic resistance genes (ARGs): 50.0- 85.8	Positive	Positive
2015-Zurita et al.	Ocotlán, Jalisco, México	HSSFCW and VSSFCW	Natural WW	2	*Strelitzia reginae, Zantedeschia aethiopica*	1	Tezontle gravel	3-5	CF/E. coli.: 99.93-99.99	Positive	Positive
2014-Zurita et al.	Ocotlán, Jalisco, México	Horizontal and vertical SSFCW	Gray Water, Sewage, Lab water	3	*Canna indica, Strelitzia reginae, Zantedeschia aethiopica,*	1	Ground tezontle rock	3	TN: 20-57 TP: 0 E. coli: 99.9	Positive	Positive
2013-Arroyo et al.	León, Spain	VSSFCW and FFP-FCW	Industrial WW	2	*Phragmites australis, Typha latifolia*	1	Light expanded clay (Arlita)	0.083 and 0.125	As: 7.8-22.8, Zn:20-55	Positive	Positive
2012-Mateus et al.	Tomar, Portugal	SSFCW	Urban WW	1	*Phragmites australis*	1	Fragmented Moleanos limestone (FML)	4	P: 61	Positive	Positive
2012-Zurita et al.	Ocotlán, Jalisco, Mexico	SSFCW	Drinking water	2	*Anemopsis californica, Zantedeschia aethiopica*	1	Ground tezontle rock	5	As: 79-92	Positive	Positive
2010-Zhao et al.	Nanjing, China	VSSFCW	Domestic WW	1	*Lythrum salicaria*	2	Gravel, granulated slag	1.5	COD:41-68, TN:24-62, TP:35-71, TOC:16-37	Positive	Positive
2009 Zurita et al.	Ocotlán, Jalisco, Mexico	Vertical and horizontal SSFCW	Domestic WW	4	*Agapanthus africanus, Anturium andreanum, Strelizia reginae, Zantedeschia aethiopica*	1	Tezontle gravel	4	COD: >80 BOD_5:>80 TP: >50 Org-N: 50.6 NH_4^+:72.2 TC: 96.9	positive	positive
2008-Zurita et	Ocotlán, Jalisco,	Vertical and	Domestic	1	*Zantedeschia aethiopica*	1	Tezontle gravel	4	COD: 78 BOD_5: 80	positive	positive

al.	Mexico	horizontal SSFCW	WW						TN: 49 TP: 41		
2006-Zurita et al.	Ocotlán, Jalisco, Mexico	Horizontal and vertical SSFCW	Domestic WW	5	*Anthurium andreanum, Canna generalis, Strelitzia reginae, Hemerocallis dumortieri, Zantedeschia aethiopica*	1	Tezontle	4	COD: >75 BOD_5:>70 TN: >70 TP: >66	positive	positive
2005-Kyambadde et al.	Kampala, Uganda	HSSF CW	Domestic WW	2	*Cyperus papyrus, Miscanthidium violaceum*	1	Gravel	2.71	BOD, NH_4^+–N and PO_4^--P: 46.7–86.5, TN:15-28.5, TP:9.3-11.2	Positive	Positive
2001-Brix et al.	Risskov, Denmark	SSFCW	Domestic sewage	1	Reed plants (*Phragmites australis*)	6	Natural sands (13 types), LECA, crushed marble, diatomaceous earth, vermiculite and calcite	0.5	TP:50 to >80	Positive	Positive
2000-Brooks et al.	Newark, USA	Upflow VSSF CW	Secondary municipal WW	0	_	1	Wollastonite	3	TP:80-96	_	Positive
1999-Tanner et al.	Hamilton, New Zealand	Subsurface flow CW	Farm dairy WW	1	*Schoenoplectus tabemaemontani*	1	Gravel (L1-L4)	1.95 - 6.54	TP: 31-55	Positive	Positive
1997-Drizo et al.	Edinburgh, UK	HSSF CW	Sewage	1	*Phragmites australis*	1	Shale	5	TP: 98–100, NH_4^+-N: 100, NO_3-N: 85-95	Positive	Positive
1996-Wood and McAtamney	Ballyronan , Northen Irland	Pilot-scale FCW	Domestic WW	2	*Phragmites australis, Phalaris arundinacea*	2	Laterite, granite gravel	4	TP:96-99 Al and Fe: 85-98	Positive	Positive
1993-Mann and Bavor	Richmond, Australia	Vertical subsu	Industrial WW	2	*Typha oriental is, Schoenoplec*	5	Regional gravel, gravel	3-4 [1st year]	P: 40, [1st year], -40 [2nd year]	No Effect	Positive [1st

		rface flow CW			*tus validus.*		substratum, granulated blast furnace slag, blast furnace slag, fly ash	, 6-8 [2nd year]			year], negat ive [2nd year]

In recent past, an increased interest in exploring various design aspects of constructed wetlands was also observed among scholars (Fig. 2.8). A total of 247 publications were found in SCOPUS database using the keywords “artificial wetland", "engineered wetland", "ecological treatment system", "wetland", "wetland biofilter” AND "design" in “title”.

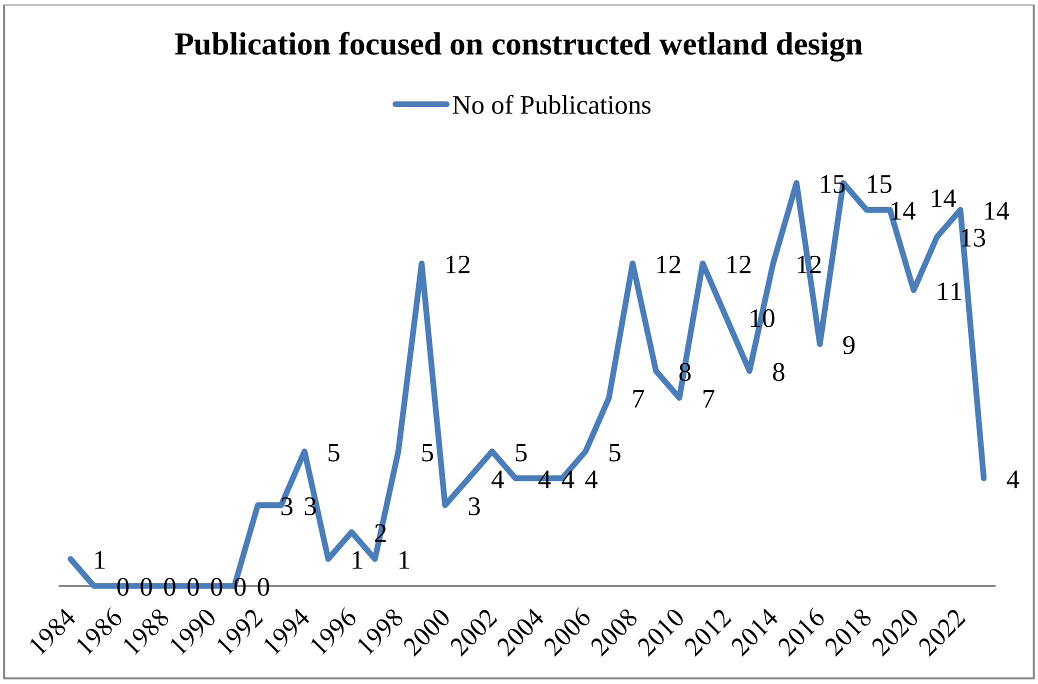
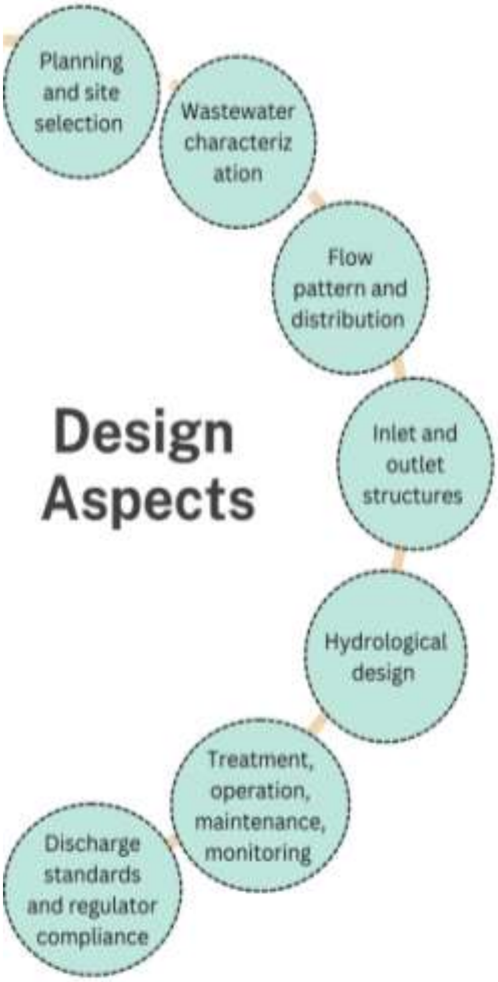


Fig. 2.8 Increase in publications focused on various design aspects of constructed wetlands

The increased number of publication shows noticeable focus on the design of constructed wetlands, with emphasis on optimizing treatment efficiency and enhancing biodiversity. The increasing recognition of constructed wetlands' efficacy in sustainable water treatment and ecological restoration is the reason for the heightened interest in their design. Because of its many advantages and minimal environmental impact, researchers and environmentalists are looking at nature-based solutions like constructed wetlands as concerns about water quality, biodiversity loss, and climate change rise.

2.9. Constructed wetland treatment system: design considerations

As design of constructed wetlands have a crucial impact on the treatment performance, various important aspects in the design process are identified (Fig. 2.9) and key considerations during each stage to achieve optimum performance are discussed. CWTS design is a multi stage process that requires careful planning and thoughtful consideration of various factors that influence the treatment effectiveness and long-term sustainability. The design of the integrated treatment unit should be oriented towards building a biological and hydrological functional system. Wetland design should be able to maintain the wetland hydraulics as loading rate and retention time has a significant influence on the overall treatment performance of the unit (Ghosh and Gopal, 2010). To design an efficient and sustainable wastewater treatment system, it is essential to employ a multidisciplinary approach that involves engineering, biology, and environmental sciences.



2.9.1. Planning Stage

The very first stage is planning which involves clearly defining the treatment objectives and setting clear goals for the constructed wetland treatment system. Wetland system can be designed for different purposes like for primary treatment focusing on removal of solids and suspended particles, for secondary or tertiary wastewater treatments targeting biological organic matter removal, for management of some specific type of wastewater or some particular contaminant. Wetland can also be established with the aim of habitat recreation and ecosystem restoration. Design of the wetland unit should be in alignment with the intended objectives. The following stage is to determine an appropriate location for the system, characterize the influent wastewater and choose the type and configuration of the constructed wetland system based on the objectives of treatment and the influent characteristics. Some of the commonly used wetland configurations are horizontal flow systems, vertical flow systems, and hybrid systems which combine both. Wetland systems may also be designed to contain multiple cells arranged in parallel or series depending on the anticipated flow rates and retention requirement. Apart for this, planning stage also involves ensuring compliance with regulatory requirements. It is essential to take into consideration all local, state, and national regulatory agencies, as well as their effluent discharge regulations when determining the effluent quality standard that needs to be met. Additionally, planning should also consider any possible future extensions of the treatment system.

2.9.2. Site selection and assessment

Selection of a suitable site and its assessment is the next stage in designing a constructed wetland treatment system. Assessment of the site involves gathering information about the local topography, site constraints, slope, soil quality, groundwater exchanges and soil quality. The selected site needs to have suitable hydraulic and geological conditions. The selected site should have adequate space for installation of wetland filter bed and any preliminary and tertiary treatment if required. Land availability is directly associated with the wetland size which influences the retention time and hence the overall effectiveness of pollutant removal. While selecting the site, due consideration should also be given to availability of land for creating a buffer zone around the wetland and accommodation of any future expansion. Often it is more convenient to install the CWTS at the source of wastewater generation as it minimizes cost incurred in collected and transportation of wastewater.

To make use of natural flow processes and promote gravitational water flow and ease drainage, many systems incorporate a slight slope in the wetland treatment cells which helps to maintain the flow velocity. This slope also helps in avoiding accumulation of pollutants in stagnant areas. Finding out the gradient and slope of the selected site can help to facilitate movement of water through the system. Site with a gentle slope can be easily modified to collect and hold wastewater and facilitates its gravitational flow through the wetland system, which further reduces need for pumps, motors and daily valve adjustments. Also, the site should be easily accessible to personnel and vehicles. Installing the CWTS far from residential communities will reduce risk of odour and insects. However, if it needs to be installed near dwellings, a buffer zone can be placed next to the wetland. Additionally, the selected site should not be located in floodplain or at risk of any specific contamination. Site should not contain any historical or archaeological resources.

2.9.3. Wastewater characterization

Characterizing the wastewater is important in order to identify the nature and concentration of the pollutants present in the wastewater. It also involves determining the quantity of the wastewater that needs to be treated. Information of the type of contaminants along with regulatory discharge standards can help to define the specific treatment objectives like removal of nutrients, organic matter, pathogens etc. Once the treatment objectives are defined, site is selected and wastewater is characterized, attention should be paid to the selection of suitable plants and substrates and hydraulic design of the treatment system.

2.9.4. Wetland specifications and component selection

Selection of appropriate type of constructed wetland is another important aspect. Choice of the constructed wetland layout and its configuration, type of flow pattern, influent loading, arrangement of cells etc is based on the type of the influent and desired treatment objectives. The length-to-width ratio for a constructed wetland treatment system should typically be between 1:1 and 4:1. A square wetland (1:1) is often most effective from the perspective of construction expenses. Plants and substrate are two major components of the wetland system. Some of the commonly used plants and substrates in constructed wetlands are listed in Fig. 2.4 and Fig. 2.5 respectively. Diverse plant species can be used to promote biodiversity, improve aesthetic appearance and enhance habitat value of the CWTS. Numerous studies have reported efficient performance of plants in monoculture and polyculture in constructed wetlands. Plants in the system needs to be arranged in such a manner that it maximizes the contact time between the wastewater and plant roots.

2.9.5. Hydraulic considerations

The term "hydraulics" refers to the flow of water through the wetland system. One of the key elements that contribute to the system's effectiveness is its hydrology. A system with an inappropriate hydraulic design may experience a number of issues that could reduce the system's productivity. The hydraulic design of the system should be tailored to the meet the specific treatment objectives and discharge standards. Hydraulic design of the CWTS includes ensuring even water distribution pattern, uniform flow paths, appropriate pollutant loading rate, adequate water retention time, preventing short-circuiting etc. Important considerations in the hydrologic design are given in Fig. 2.10

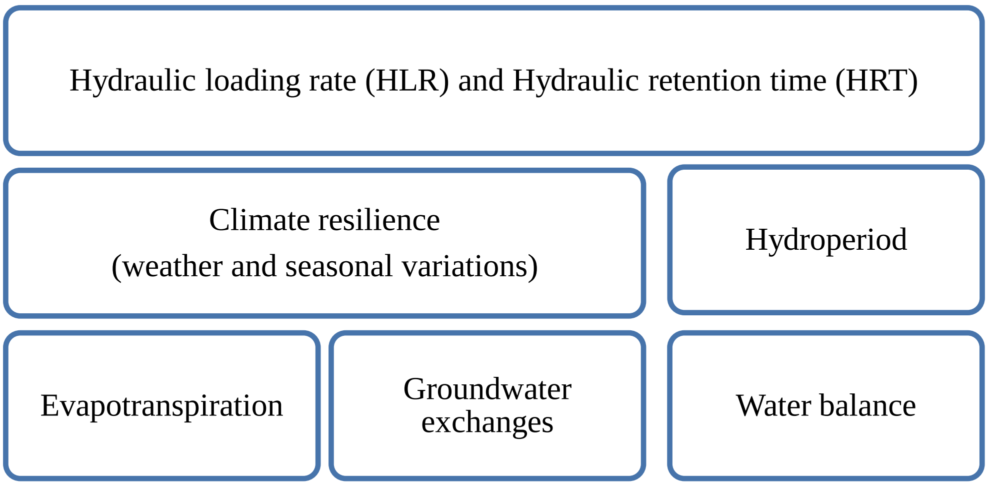


Fig. 2.10 Important hydrologic considerations in the wetland design process

2.9.5.1. Hydraulic loading rate (HLR) and hydraulic retention time (HRT)

HLR is the amount of water applied to a unit area of the wetland in a given amount of time. HLR is crucial for sizing the wetland and striking a balance between the wetland's capacity for treatment and the available area. HLR is calculated using the formula

$q = Q/A$

Where, q is hydraulic loading rate (m/d),

A is wetland area (m^2)

Q = water flow rate (m^3 / d)

Another crucial factor that influences the extent of pollutant removal is the hydraulic retention time, which is the length of time for which water remains in a wetland system. It is usually based on the treatment objectives, water flow rates, and pollutant loading rates. Hydraulic retention time (HRT) is determined by the flow depth and the mean surface area of the system. It is also influence by the space available for the water to move across the substrate bed and plant roots.

2.9.5.2. Climate, weather and seasonal variations

Wetland treatment systems are open structures that are strongly influenced by the changes in weather and climate. Events like heavy rainfall, rapid snowmelt, and spring runoff can cause high flow rate and excessive water in the wetland which shortens the resident duration and contact time thereby reducing the efficiency. Excess runoff may require diversion to avoid overflow through the wetland unit. Moreover, incorporation of overflow and emergency spillway structures in the wetland unit can help to protect the integrity of the wetland, avoid floods and handle the excessive water flow received during heavy rainfall and to prevent flooding. Furthermore, some free space or a safety buffer zone over the maximum water level can also be provided to account for unforeseen changes in the water level. Wetland should be designed to handle the fluctuations in the influent water quality, pollutant load and account for adverse climatic conditions like drought and rainfall. In extremely cold regions surface freezing can prevent entry of water into the wetland unit. Measure to prevent freezing of the filter bed surface man be required in winter months and in colder regions. Change in temperature also influence the microbial processes involved in breakdown of pollutants.

2.9.5.3. Hydroperoid

Hydroperoid is another critical aspect of wetland ecology and design. It refers to the availability of water throughout the year and temporal pattern of fluctuations in water level, which is strongly affected by the seasonal variations in precipitation and evapotranspiration. It is the outcome of equilibrium between water input, outflow, and storage that affects water depth in the wetland. CWTS can face high water levels during wet phase and low water levels during dry phase. Water depth impacts the survival of wetland vegetation and hence the nutrient uptake processes. Apart from this, it also influences dissolved oxygen levels, activity of microbial communicates and redox conditions in the system. Longer hydroperoids typically result in longer retention times, enabling more treatment.

2.9.5.4. Evapotranspiration and groundwater exchange

Evapotranspiration (ET) refers to the combined water loss through evaporation from wetland's surface into the air and through plants transpiring out of their leaves. It is a natural component of water balance. In order to maintain the wetland's water balance and prevent the concentration of pollutants at harmful levels, additional water will be required if ET losses exceeds water inflows. CWs are often lined to prevent any groundwater contamination. As long as the wetland is adequately sealed, infiltration should be minimal.

2.9.5.5. Overall water balance

The overall water balance of a CWTS is influence by the water inflow into the systems i.e. the wastewater entering the system, precipitation and groundwater infiltration in case the wetlands are not sealed. Another component of the water balance is storage water which includes surface water and water in the pore spaces of the filter bed. Lastly outflow water also influences the overall water balance. Outflow water includes transpiration by plants, discharge of treated water and evaporation from the water's surface.

For a constructed wetland, the standard water balance equation can be expressed as

$S = Q + I - O$

Where: S = net change in storage

Q = Inflow water (surface flow + wastewater + storm water+ rainfall water)
I = net infiltration (infiltration - exfiltration)
O = surface outflow (transpiration + effluent discharge + evaporation)

2.10. Inlet and outlet structures

Inlets structures are usually made up of pipes, weirs, valves, distribution channels with flow control devices to regulate the flow rates and distribution of the influent. To avoid preferential flow paths sometimes flow splitting using bafflers and distribution channels along with equalization using separate is employed to achieve uniform flow distribution across the wetland bed. It also involves calculating the desired flow rates and velocities as it have an influence on the filter bed and the treatment performance. For instance, excessive water flow could lead to erosion and can interfere with the plant growth. It is significant to control the influent flow patterns and distribution to regulate the flow path of the influent through the substrate. Influent can be made to flow horizontally, vertically or in a combination of both patterns. This usually depends on the wetland area, influent characteristics and treatment objectives.

2.11. Influent flow patter and distribution

As the type of contaminants and pollutant levels can greatly fluctuate in wastewater therefore it is advisable to firstly equalize or balance the flow rate and organic load of wastewater before it enters the wetland bed. Different types of flow equalization tanks that offer alternative flow diversion, intermittent flow diversion or completely mixed combined flow systems can be utilized to regulate the variability of flow and level of pollutants in the influent. While designing the flow arrangement attention should be given to minimize short circuiting of wastewater between the inlet and outlets and limiting any stagnant pools or dead zones in the corners. Thus, designing the inlet and outlet structure control systems is also critical to provide an effective flow distribution and maximize the frictional resistance. When water is dispersed across complete area as opposed to being contained within a channel, frictional resistance is higher.

To ensure even flow distribution in the inlet zone, the bottom slope should be practically zero. The inlet structure can be made up of gated pipe or ungated gravity overflow pipes that can release wastewater into the wetland bed. During design it should be ensured that the inlet and outlet structures are accessible and easily adjustable with easy access for monitoring the flow, taking sample for analysis and routine maintenance. Outlet zones are vulnerable to debris build up and algal growth. To limit biomass export, final filtering of the algae biomass in the wetland is preferred. Aquatic plants should be used for final filtering in the system configuration. Other options include using a rock filter or set up a large-mesh debris fence a few meters away from the outlet structure. To collect and channel flow to the outlet weir, a deep open water zone can be created. To avoid long water residence time and subsequent algal growth, this terminal open zone needs to be kept small. Also, the ultimate discharge point from the wetland system should be situated high enough above the receiving water so that a rise in the receiving water's level, such as after a storm, won't obstruct the flow of water through the wetland. Water level can be regulated by a weir, spillway, or adjustable riser pipe that might serve as the wetland's outlet structure. Operating and maintaining the wetland can benefit significantly by making use of an adjustable outlet, which maintains an appropriate hydraulic gradient in the bed.

2.12. Operation, monitoring, maintenance and regulatory compliance

To ensure the wetland's efficient performance and long run, regular monitoring, inspections, and maintenance are crucial. Routine operations include water quality assessment, sediment removal, managing the vegetation, controlling invasive species, and periodically harvesting above ground biomass. Regular inspections and assessment of the wetland unit is essential to ensure optimal performance. Sampling points should be place in locations that are easily accessible for sample collection and analysis. Design should also account for easy equipment placement and adjustments as needed. The design should provide convenient access for inspecting water levels, regulating water flow, examining and controlling flow path, harvesting plants and performing other routine maintenance activities. Various sensors can be installed to check water level, temperature and pH of the system. Flow meter can also be used to monitor the flow rate at different points.

2.13. Stakeholder engagement

Getting advice and recommendation of wetland design professionals, engineers, hydrologists, ecologists, environmental agencies and environmental consultants throughout the design process can help to create more efficient wastewater treatment systems. Participation and support of the local communities and all stakeholders can boost acceptance of the treatment system, its routine maintenance and long term operation. Educational and other outreach initiatives can be planned to inform the community and individuals regarding the CWTS and their ecological benefits.

2.14. Risk assessment, safety and environmental consideration

While designing CWTS, due attention should be given to identification, evaluation and mitigation of potential risks that might influence sustainability of the CWTs. Various ecological, regulatory, financial and operational risks associated with the use of CWTS are listed in Fig.2.11 Prioritizing the identified risks, categorizing its impact (low, moderate, and high) and assigning severity rating to each can help to design appropriate mitigation efforts to address then effectively. Mitigation strategies are aimed to minimize disturbances and can include modification of design for examples incorporating overflow and emergency spillway to manage excessive water during rain. Ensuring regular inspections to check spread of any invasive species or insects. Regular assessment of pollutant load and effluent quality also needs to be ensured. Design process should also involve developing standard procedures for responding to events like sudden weather change, flooding, equipment failure etc. Contingency plan for unexpected influent contaminants and pollutant load should be prepared.

Financial and Operational Risks

- Project budget overruns
- Funding shortfall
- Potential revenue sources
- Obtaining the necessary permits
- Ensuring regulatory compliance
- Equipment failure
- Long term maintenance costs

Environmental Risks

- Breeding ground for insects
- Impact on local flora and fauna
- Soil erosion
- Flooding risks
- Transmission of antibiotic resistance genes
- Contaminant leaching
- Impact on adjacent ecosystems

Fig. 2.11 Risks associated with constructed wetland treatment systems

Apart from allocating resources for routine maintenance, some fund should be kept for potential corrective measures that may be required from time to time. Incidences if any needs to be properly documented along with the information of the corrective actions taken so that the records can serves as reference for future. The system needs to be adaptable and responsive to the changing environment.

The wetland design and treatment performance should comply with the local, regional and national regulations governing the water quality, discharge standards and environmental protection measures. Required permission needs to be obtained before the treatment setup and all date needs to be maintained for regular inspections and necessary approvals/permits from time to time.

Appropriate measures should be adopted to address issues like changes in flow pattern, extreme weather events, contaminant leaching from substrate, soil erosion, transmission of antibiotic resistant organisms etc (Zhang et al., 2024). Any impact on the local flora and fauna should be assessed. Soil type, quality, stability and potential for erosion should also be checked. There are certain risks associated with the selection of plant species as well for examples invasive weeds and loss of vegetation cover. Control measure should also be planned for the risk associated with conflict with wildlife, breeding of insects, pests and other invasive species.

Furthermore, in case, wetland system is located near residential area a buffer zone can be created. Buffer Zone can also help to protect against potential damage. A buffer zone around the wetland can consist of shrubs, grasses or other native plant species. Stabilizing area around the wetland with vegetation helps to prevent soil erosion. Additionally, fencing can be used around the wetland to prevent unauthorized access. Guardrails and warning signs can be placed at appropriate and visible locations to avoid any damage that can be caused by animals and humans.

Considering the extensive review of literature on constructed wetland component selection and design aspects, it was observed that most of the initial studies were carried out using weeds like *Phragmites australis* (Vymazal, 2011). For tropical climate, these plants are not native. Moreover, the potential invasive behaviours and less commercial value don't make these plants a good choice for wetland systems. Even today the majority of studies use *Phragmites australis, Typha, Schoenoplectus,* etc. which is not a very good choice due to its invasiveness and less commercial value (Latune et al., 2017). Additionally, mostly natural materials like soil, sand, and gravel were utilized as traditional filter materials in artificial wetlands. However, these filters are not much efficient in nutrient removal. In recent years many different natural and artificial/modified substrates having improved adsorption capacity have been evaluated as components of the constructed wetland substrates (Shen et al., 2020). Various natural and artificial materials as components of wetland media have been investigated over the years; however, the influence of agricultural waste as wetland media, on the treatment efficiency of the system is rarely documented.

There is a need to explore alternative substrates having good sorption capacity and ornamental plant species having good stress tolerance, remediation potential, and some commercial value in constructed wetland systems. Using different agricultural materials and biomaterial bio-products, biochar is a potential research topic to be investigated in wastewater treatment using constructed wetlands (Saba et al., 2015). Hence, the present study was undertaken to explore various organic materials as substrates for the growth of ornamental plants in constructed wetlands. Additionally, various design aspects that influence treatment performance are identified and key considerations are proposed.

Chapter 3

Objectives:

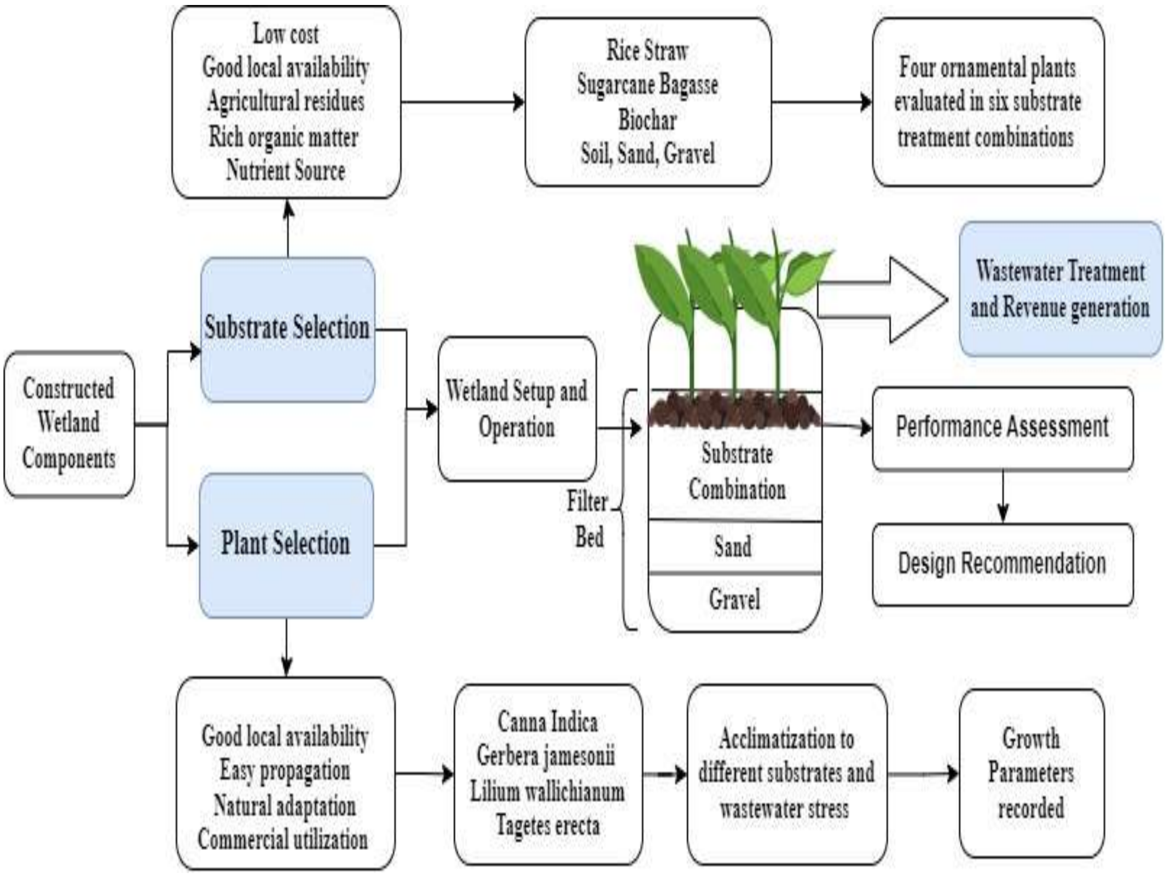
1. Selection and screening of suitable substrates and plants for the wetland system
2. Comparison of treatment efficiencies of different combinations
3. Design and development of vegetative wetland system

Chapter-4

Methods and materials

4.1 Location and site description

This study was conducted in a green house to investigate the potential of agricultural residues as a wetland substrate on the growth of ornamental plants in constructed wetland at Lovely Professional University (LPU), Punjab, India (latitude 31.2560° N, longitude 75.7051° E, altitude 234 m) from December 2020 to June 2021 using agricultural residues abundantly available in the Punjab region. Mesocosm constructed wetlands were installed in the shaded net of the Department of Agriculture, green house, located at LPU. Schematic diagram of the methodology used in the study is presented below.



Scheme 4.1: Methodology used for selection of wetland components, plant growth evaluation and treatment performance assessment

4.2 Constructed wetland media used

Besides the conventional wetland substrates (soil, sand, and gravel), incorporation of locally available agricultural and industrial waste in the media, as an organic matter is known to provide more carbon, promote the growth of microbes, and also increase nutrient absorption (Ji Z et al., 2022). The state of Punjab is one of the major agricultural growing areas in India. Some of the important crops grown in Punjab are rice, wheat, and sugarcane which result in surplus crop residue in the field after harvest. In order to get ready for the next plant growing season, farmers prefer to burn this excess residue in the field rather than mulching it into the soil with a tillage tool due to lack of affordable agricultural mulching equipment.

Rice straw left in the fields and sugarcane bagasse discarded by sugarcane factories after extraction of juice are the two most abundantly available agricultural by-products in Punjab. In the present study rice straw and sugarcane bagasse in combination with locally available soil were used as substrates for plant growth (Fig. 4.1). Further, 5 % biochar, which is a stable carbon-rich material produced from the thermo-chemical decomposition of biomass was also added into the substrate media in all treatments except one. Biochar plays an important role in the improvement of soil fertility, environmental remediation, and carbon sequestration. Biochar is also known to promote microbial growth and provide a large surface area for the adsorption of pollutants (Wang H et al., 2020).

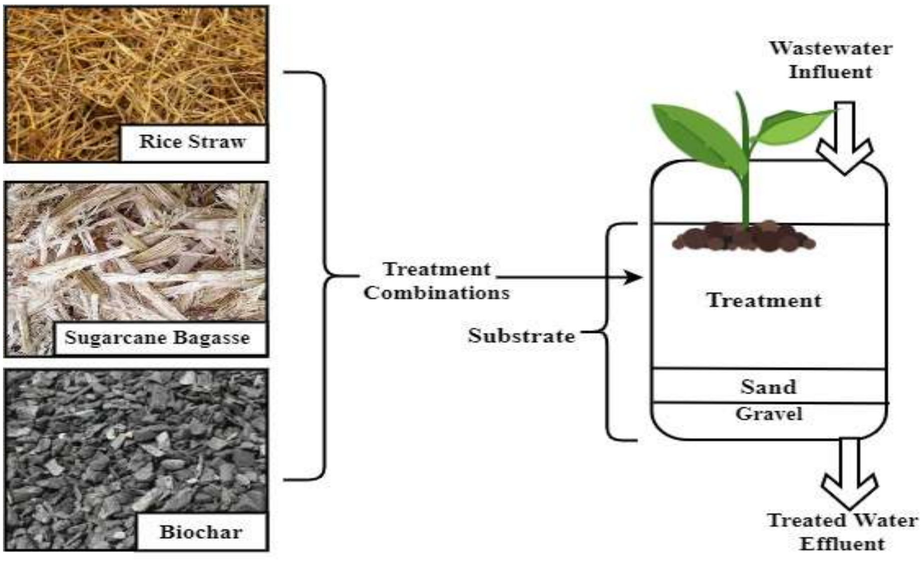


Fig 4.1: Incorporation of agricultural residues and biochar as substrate components in constructed wetland filter bed (Sharma et al., 2023)

4.3 Ornamental plants used

The majority of constructed wetland studies found in the literature used plants like *Phragmites australis, Typha*, and *Schoenoplectus* which do not have any good commercial value in the market (Vymazal, 2018). The economic potential of using ornamental plants in constructed wetlands has recently been realized (Marín-Muñiz et al., 2023). Ornamental plants have excellent commercial value but only a few studies have been conducted on the growth performance of ornamental plants (Calheiros et al., 2015). Therefore, we selected four ornamental plants for the present study. Plants were selected based on three criteria: (i) good local availability, (ii) commercial utilization in the local markets, and (iii) easy propagation and adaptation to the natural environment. Hence, *Canna indica* (P1), *Lilium wallichianum* (P2), *Tagetes erecta* (P3), *and Gerbera jamesonii* (P4) were selected to be grown in the mesocosms containing six different treatment combinations in triplicates. *Lilium wallichianum* and *Tagetes erecta* have not been used previously in constructed wetlands.

Young sprouts of the plants (*Canna Indica, Gerbera Jamesonii, Lilium Wallichianum, Tagetes erecta*) of the same height (3-4 inches) were collected from LPU greenhouse and a local nursery and planted in the mesocosms containing different media in the month of February 2021. For three months plants were allowed to establish, multiply, develop into dense strands, and adapt to the wastewater stress and water logging conditions of the mesocosms before the treatment performance was evaluated. Wastewater was stored in a 500 L plastic tank in the dark for not more than one week. For initial 30 days of the experiment, mesocosms were irrigated with tap water followed by irrigation with wastewater for the next 60 days. Management practices like irrigation, weeding etc. remained same for the all treatments.

Details of the ornamental plants used are provided in Table 4.1. The stages of growth of the plants during the study period are given in Table 4.2.

Table 4.1: Ornamental plants used in the study (Sharma et al., 2023)

Sr No	Family	Botanical name	Plant Common Name	Plant	Flower
1	Cannaceae	*Canna indica*	Indian shot, wild canna lily, canna		
2	Asteraceae	*Gerbera jamesonii*	Barberton daisy, Transvaal daisy, African Daisy		
3	Liliaceae	*Lilium wallichianum*	Lily		
4	Asteraceae	*Tagetes erecta*	African Marigold		

Table 4.2: Growth stages of the selected ornamental plants

Sr No	Plant Species	Month				
		February	**March**	**April**	**May**	**June**
1	*Canna indica*	i	ii	ii	iii	iii
2	*Gerbera jamesonii*	i	ii	iii	iv	-
3	*Lilium wallichianum*	i	ii, iii	iv	-	-
4	*Tagetes erecta*	i	ii	iii	iii	iv

Note: Stages of plant growth

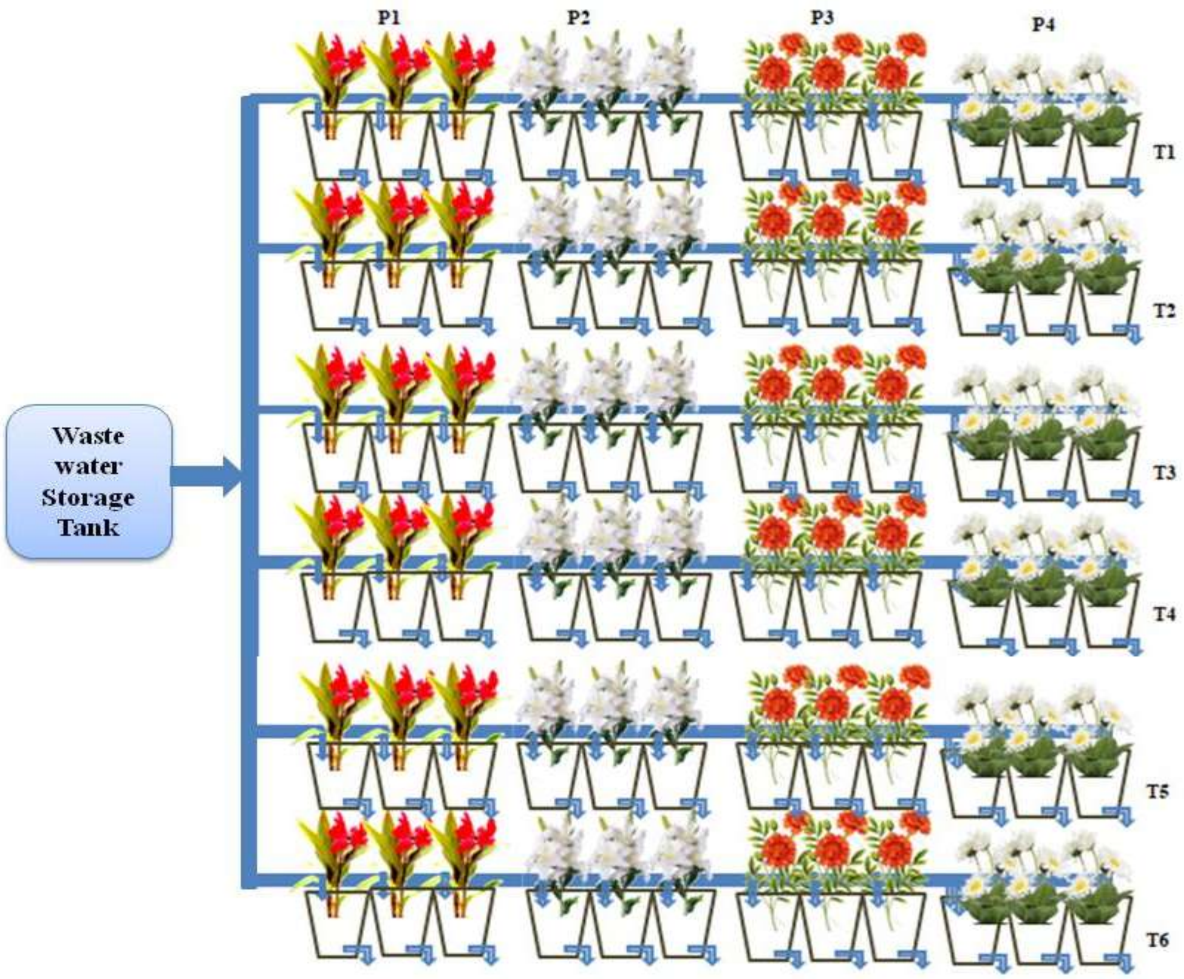
i. Plantation stage- Initial stage of plantation i.e. from seedling to stem elongation
ii. Adaptation stage- Stem elongation to the initial flowering stage
iii. Development stage- Growth and flowering stage
iv. Late-season stage- From initial senescence to plant harvest/death

4.4 Experimental design and mesocosms setup

The two-factor experimental design was used in the study where one factor was the media type and the other factor was plant species. The study was conducted in two phases: in the first phase, ornamental plants were planted in mesocosm and observed for their growth using different substrate combinations. Four plants were grown on six substrate treatment combinations for five weeks to select the best plant and substrate for the study. In the second phase, wastewater was added into all units to observe plant acclimatization and treatment efficiency of different plant substrate combinations for the removal of organic matter and nutrients from wastewater.

Collection of the raw materials such as plastic pots, substrate, stands etc. was completed in the month of January 2021. The preparation of the treatment combination and mesocosms setup was assembled in the month of February 2021. Rice husk and sugarcane bagasse were collected from local farmers, air-dried, chopped into small pieces (1-2 inches), and mixed homogeneously to obtain the required treatments. Biochar was crushed into fine powder and used in the substrate mix at 5% of the total volume in each of the treatment.

Each mesocosms unit comprised 6 L, 10 L, and 15 L plastic containers filled with a bottom layer of sand and gravel. Above that, other media constituents i.e. soil, biochar, and lignocellulosic waste were added. The top 4 cm space was kept as a free surface. Table 4.3 shows the details of the six treatments used in the study. Treatment, T1, with 100% soil in the pot was used as a control treatment. Treatment T2 was having 95% soil and 5% biochar to evaluate the effect of biochar on plant growth. Two treatments having rice straw (T3, T4) and two treatments having sugarcane bagasse (T5, T6) were used in the study. 5% biochar by volume was kept constant for five treatments (T2 to T6). The study was having a total of one control (T1) and five treatments (T3, T4, T5 and T6). Pots having six substrate treatment combinations in triplicates were prepared for four ornamental plants resulting in a total of 72 pots. A schematic diagram of the experimental design and mesocosm setup is presented in Scheme 4.2 and Fig. 4.2 respectively.



Scheme 4.2: Experimental design showing ornamental plant species (P1 to P4) grown in mesocosms filled with different types of media (T1 to T6) and irrigated with wastewater

Table 4.3: Substrate combinations used for six treatments

Sr. No	Treatments	Substrate Used	Substrate Combinations	Components (%)			
				Soil	Rice Straw (RS)	Sugarcane Bagasse (SB)	Bio char
1.	T1	Soil	Soil (Control)	100	-	-	-
2.	T2	Soil, Biochar	Soil+ Biochar	95	-	-	5
3.	T3	25% Rice Straw	Soil+ RS+ Biochar	70	25	-	5
4.	T4	15% Rice Straw	Soil+ RS+ Biochar	80	15	-	5
5.	T5	25% Sugarcane Bagasse	Soil+ SB+ Biochar	70	-	25	5
6.	T6	15% Sugarcane Bagasse	Soil+ SB+ Biochar	80	-	15	5

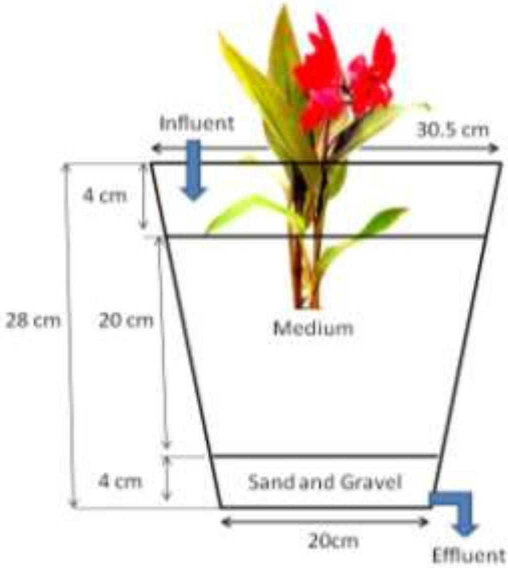


Fig. 4.2: Mesocosm setup used in the present study

Plantation was done in the month of February and plants were allowed to establish themselves in the pots for the first two weeks and then were observed for different plant growth parameters. Pots were irrigated with tap water initially once every three days for the first 2 months (February and March) after planting. Irrigation with wastewater was started in the month of April. Wastewater used for irrigation in this study was collected from a drain passing through the campus of Lovely Professional University. In April irrigation was done once every two days and for the months of May and June, pots were irrigated once daily with wastewater. Table 4.4 provides details of the pot volume used and the amount of substrate and wastewater added in each treatment. Since the composition of substrates is different in each treatment, inaccuracies may arise in the volume of water to be used for irrigation in each treatment. Therefore it is important to calculate the volume of the wastewater to be added to each treatment type separately.

Table 4.4: Details of the pot volume, substrate weight, and wastewater added in each treatment

Treatment	Total Pot Volume (L)	Substrate Weight (Kg)	Wastewater Volume Added (L)	Total (Substrate weight+ Wastewater added) (Kg)	Volume of water drained (L)
T1	6	3.79	1.5	5.29	0.7
T2	6	3.87	1.5	5.37	0.6
T3	15	2.67	10	10.26	3.2
T4	15	4.46	8	12.46	2.3
T5	10	1.99	6	7.99	1.9
T6	10	2.96	5	7.96	1.5

4.5 Wastewater characterization

Wastewater used in the study was collected from a drain passing through the University campus. Wastewater characteristics were analyzed in the Bioengineering and Biosciences Laboratory for pH, Total Dissolved Solids (TDS), 5-day Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Phosphorus (PO_4-P), Ammonia (NH_4-N) and Nitrate (NO_3-N). Characterization of the wastewater (Table 4.5) was carried out for consecutive 15 days before starting the operation period, as per standard methods for the examination of wastewater by the American Public Health Association (APHA 2017) using a digital pH meter (LT-50, Labtronics, India, accuracy: ± 0.01 pH ± 1 digit), TDS meter (HM Digital, range: 0-9990ppm, accuracy: ±2%), BOD incubator cum shaker and COD digester (Lablink, Bombay Scientific). The pH and TDS of the influent varied from 7 to 8 units and 515 mg/L, to 638 mg/L respectively. BOD was found to vary from 84 mg/L to 154 mg/L with an average value of 233 mg/L which is similar to those reported by Singh et al. (2022) who worked with the same influent. COD values ranged from 157 mg/L to 264 mg/L with an average value of 334 mg/L.

Table 4.5: Physiochemical characterization of wastewater used in the study

Parameter	Value	Minimum	Maximum
pH	7.12-8.34	7.12	8.2
TDS	663.26±77.70	515	638
Biochemical Oxygen Demand	233.07±62.26	84.26	154.17
Chemical Oxygen Demand	334.33±71.53	157.08	264.18
Ammonia	35.95±6.91	25.68	48.76
Phosphorus	6.58±1.23	4.68	8.43
Nitrate	12.34±3.80	6.12	18.3

Parameter units- mg/l except for pH (n=15)

4.6 Measurement of plant growth parameters

Following plant growth parameters were monitored in the study:

i. Plant height: The height of each plant was measured using a measuring tape in cm from the base to the top leaf of the plant.

ii. Number of leaves: The total number of leaves per plant was counted every week for each plant.

iii. SPAD Unit- A chlorophyll meter SPAD-502 Plus of Konica Minolta, having accuracy within ±1.0 SPAD unit was used to measure the amount of chlorophyll present in the leaves. Three reading were taken for 3-5 leaves and an average was used.

iv. Number of flowers: Flowers per plant were counted weekly for each plant.

v. Days to flower emergence: The number of days was counted from the date of sowing to the emergence of the first flower on the plant.

vi. Stem Thickness: Stem diameter was measured by wrapping the stem with a thread at three points and taking readings on the scale. An average of three values was used for stem thickness for all plants.

vii. Microscopic examination- A stem cross-section was examined under the Magnus MLX-DX Microscope (Olympus, India) and captured using Magnus live USB2.0 Viewer Software Version 1.1.2.3, to check cell wall organization and tissue structure. Leaf peels were also visualized to check stomata.

viii. Root morphology- Root systems of the plants were observed to gain a better understanding of functions like nutrient uptake and water absorption.

ix. Biomass- Above and below substrate level plant biomass, along with plant fresh and dry weight were also observed.

x. Elemental Analysis- The uptake of various elements like nitrogen, phosphorus, potassium, calcium, iron, etc in plant leaves was also examined per manual on soil, plant, and water analysis (Dhyan et al., 2005).

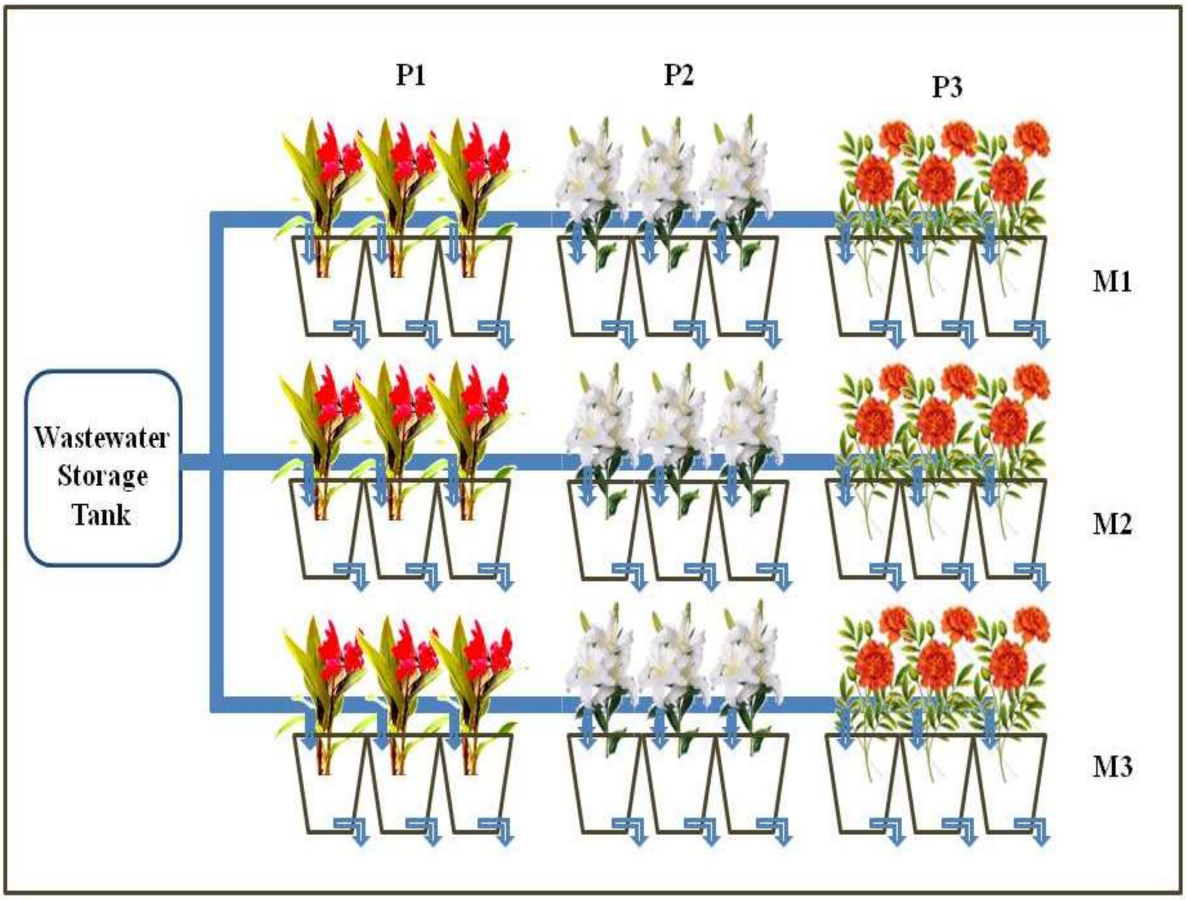
Plant growth parameters were examined to understand the development of plants in the designed system. Along with physical growth parameters, photosynthetic activity and visual stress symptoms like leaf color and wilting symptoms were also observed. The growth of ornamental plants was monitored on a weekly basis for a total of 120 days.

4.7 Performance assessment of the treatment system

Prepared substrate treatment combinations were found suitable for flowering in ornamental plants which is an indication of the availability of nutrients like phosphorus, which plays an important role in flowering, in the substrate matrix. Flowering was observed in all plants, except *Gerbera jamesonii* which was not able to adapt and produced no flowers. The performance of constructed wetland mesocosms having *Canna indica*, *Lilium wallichianum*, and *Tagetes erecta* grown in media containing organic components was further evaluated and compared for organics and nutrient removal efficiency in the second part of the study. The plants and media used for performance analysis are presented in Table 4.6 and the experimental design is presented in Scheme 4.3. Three different plant species were established in three different media combinations in mesocosm scale wetlands. Operational conditions of the vertical sub-surface flow CW mesocosms are provided in Table 4.7.

Table 4.6: Media types, composition, and ornamental plants used in the study

Media	Composition	Plants	Names
M1	Gravel, Sand, Soil, Biochar	P1	*Canna indica*
M2	Gravel, Sand, Soil, Biochar, Rice Straw	P2	*Lilium wallichianum*
M3	Gravel, Sand, Soil, Biochar, Sugarcane Bagasse	P3	*Tagetes erecta*



Scheme 4.3: Experimental design showing three different ornamental plant species (P1, P2, and P3) grown in mesocosms filled with three types of media (M1, M2, and M3)

Table 4.7: Mesocosm specifications and operational conditions

Parameter	Details
Length	28 cm
Diameter	Top- 30.5 cm, Bottom- 20 cm
Water Depth	24 cm
Volume	15 L
Vegetation	*Canna indica, Lilium wallichianum, Tagetes erecta*
Media (Substrates)	Gravel, Sand, Soil, Biochar, Rice Straw, Sugarcane Bagasse
Hydraulic Retention Time	24 hours

4.8 Characterization of influent and effluent

During the treatment operation phase, wastewater was fed from the top and was collected through a faucet at the bottom of the mesocosm after a hydraulic retention time (HRT) of 24 hours. The samples were collected once a week, from each mesocosm unit, in 500 ml high-density polyethylene bottles and transferred immediately to the laboratory for analysis and stored under refrigeration at 4ºC. The influent and the collected effluent were analyzed for total dissolved solids (TDS) using HM digital (range: 0-9990ppm, accuracy: ±2%), pH using digital pH meter LT-50, Labtronics, India (accuracy: ± 0.01 pH ± 1 digit), 5-day biochemical oxygen demand (Method 5210; APHA, 2017), chemical oxygen demand (Method 5220; APHA, 2017), phosphorus using ascorbic acid method (Method 4500-P; APHA, 2017), nitrogen forms i.e. ammonia using phenate method (Method 4500-NH_3; APHA, 2017) and nitrate using UV spectrophotometric screening (Method 4500-NO_3^-; APHA, 2017).

The pollutant removal efficiency was calculated using the formula

Percentage Removal= [(Concentration of pollutant in influent- Concentration of pollutant in effluent)/ Concentration of pollutant in influent]*100

4.9 Statistical analysis

Statistical analysis of the data was conducted using one way analysis of variance (ANOVA) using GraphPad Prism 5 with significance level of $P<0.05$.

Chapter 5

Results and Discussion

5.1. Selection of suitable substrate and plants for the wetlands treatment system

5.1.1. Physical and chemical characteristics of soil

Soil used in the study was collected from LPU (green house). Soil characterization was done in the Soil Science Laboratory of the Department of Agriculture, as per manual on soil, plant and water analysis (Dhyan et al., 2005). Physiochemical parameters of the soil were measured in the month of February 2021 and May 2021 and are given in Table 5.1.

Table 5.1 Physiochemical parameters of soil before and after irrigation with wastewater showing elemental uptake

Sr. No	Parameter	Range	Initial Value (February 2021)	Final Value (May 2021)
1.	pH	6.5-8.7	8.20	8.5
2.	EC mm hos/cm	<0.80	0.19	0.4
3.	Organic Carbon (%)	0.40-0.75	0.28	0.28
4.	Phosphorus (ppm)	5.0-9.0	6.14	23.38
5.	Sulphur (ppm)	10	9.00	19.12
6.	Potassium (ppm)	55-135	54.59	104.1
7.	Calcium (ppm)	300	223.30	201.6
8.	Magnesium(ppm)	120	145.60	130
9.	Iron(ppm)	4.50	3.88	10.31
10.	Cooper (ppm)	0.20	0.63	1.3
11.	Manganese (ppm)	3.50	0.45	5.95
12.	Zinc (ppm)	0.60	0.63	3.86
13.	Boron (ppm)	0.50	3.54	2.05

The pH values of the soil in the pots increased slightly from 8.2 to 8.5 and electrical conductivity (EC) values increased by more than twice i.e. from 0.19 mmhos/cm to 0.4 mmhos/cm. Incorporation of biochar having pH of 7.9- 8.2 might have resulted in an increase in soil pH value. Field application of wastewater is known to increase electrical conductivity and available nutrient content of the soil (Khurana and Singh, 2012). Twofold accumulation of sulphur and potassium was observed and fourfold increase in phosphorus was observed in the substrate between the months of February and May 2021 indicating the potential of substrate materials in absorbing pollutants from the wastewater.

5.1.2. Physical and chemical characteristics of biochar

Biochar used in present study was prepared by pyrolysis of the woody biomass of *Prosopis juliflora*, one of the most widespread hyper-accumulating plants, at 400ºC - 500ºC in a low oxygen environment. Table 5.2 represents the physical and chemical properties of the biochar used in the study. According to the manufacturer biochar used in the study was having following specification

Table 5.2 Physiochemical parameters of biochar used in the study (values are the mean of three representative samples)

Sr. No	Parameter	Value
1.	Moisture (%)	1.5-2.2
2.	Ash (w/w)	1.4-1.9
3.	Mobile Matter (g/kg)	38-45
4.	Residual Matter (g/kg)	31-36
5.	pH	7.9- 8.2 (1:10 solid water suspension)
6.	EC (dSm-1)	1.4-1.5 (1:10 solid water extract)
7.	CEC (c mol/kg)	16-18
8.	Organic Carbon (g/kg)	715-725
9.	Calorific Value (Kcal)	7.8-7.9
10.	Total Nitrogen (g/kg)	1.6-1.9
11.	C:N Ratio	382-446
12.	Total Phosphorus (g/kg)	1.9-2.1
13.	Total Potassium (g/kg)	24-26
14.	Calcium (g/kg)	11-13
15.	Magnesium, g/kg	0.45-0.51

5.1.3. Physical and chemical characteristics of wastewater

Wastewater used in the study was collected from a drain passing through University campus. Characterization of wastewater presented in Table 5.3 was performed as per standard methods for examination of wastewater by American Public Health Association (APHA, 2017) using digital pH meter (LT-50, Labtronics, India, accuracy: ± 0.01 pH ± 1 digit), TDS meter (HM Digital, range: 0-9990ppm, accuracy: ±2%), BOD incubator cum shaker and COD digester (Lablink, Bombay Scientific).

Table 5.3 Physiochemical properties of wastewater used for irrigation

Sr. No.	Parameter	Unit	Value
1.	pH	-	7.65±0.39
2.	Total Dissolved Solids	mg/L	826.33±34.54
3.	Total Suspended Solids	mg/L	809±42.94
4.	Biochemical Oxygen Demand	mg/L	203.16±23.92
5.	Chemical Oxygen Demand	mg/L	235.65±28.93
6.	Alkalinity	mg/L	225.66±21.4
7.	Hardness	mg/L	420.16±20.62

In the first part of the study, four ornamental plants were grown in plastic containers filled with conventional media (soil, sand, and gravel) amended with agricultural residues and biochar. Fig 5.1 shows the plants grown in the system that were observed for various growth parameters.

Fig. 5.1: Four ornamental plants established in six treatment combinations

Location- Lovely Professional University (LPU) Greenhouse, Punjab, India

(latitude 31.2560° N, longitude 75.7051° E, altitude 234 m)

5.1.4. Plant growth observations

Fig. 5.2 demonstrates the changes observed in plant height, number of leaves, SAPD unit and stem diameter of the four ornamental plants grown in six treatments during the experimental period.

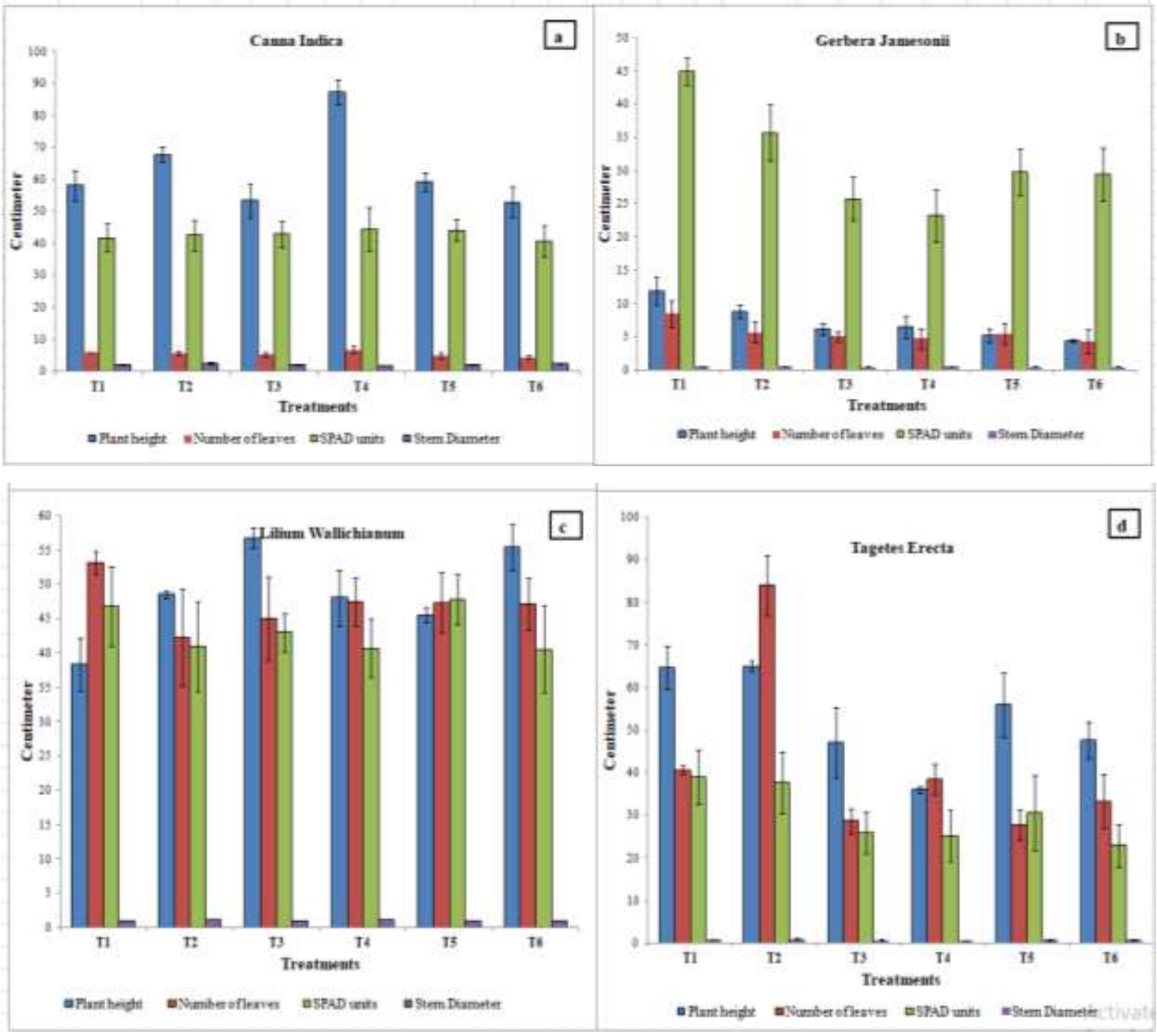


Fig. 5.2 Observed plant height (cm), number of leaves, SPAD units, and stem diameter (cm) of (a) *Canna indica*, (b) *Gerbera jamesonii*, (c) *Lilium wallichianum*, and (d) *Tagetes erecta* grown in six treatments during the experimental period.

X-axis units: Centimeter (cm) - for plant height and stem diameter (not applicable for number for leaves and SPAD units)

5.1.4.1. Plant height

The observed height of the four ornamental plants grown in six treatments during the study is presented in Table 5.4 in descending order.

Table 5.4 Plant height indicating overall plant growth and biomass yield

Plant Species	Plant Height
Canna indica	T4 (87cm)> T2 (67 cm)> T5 (59 cm)> T1 (58 cm)> T3 (53 cm)> T6 (52 cm)
Gerbera jamesonii	T1 (12 cm)> T2 (9 cm)> T4 (6.4 cm)> T3 (6 cm)> T5 (5 cm)> T6 (4.4 cm)
Lilium wallichianum	T3 (56 cm)> T6 (55 cm)> T2 (48.5 cm)> T4 (48 cm)> T5 (45.5 cm)> T1 (38 cm)
Tagetes erecta	T2 (65 cm)> T1 (64 cm)> T5 (56 cm)> T6 (47.5 cm)> T3 (47 cm)> T4 (36 cm)

Canna indica is a perennial flowering plant that grows abundantly in humid tropical regions. *Canna indica* plants are a popular choice for wetland systems as these are hardy plants with high tolerance to pore clogging and ability to survive in diverse conditions. *Canna indica* was the largest plant grown in the study. In Canna, the maximum plant height recorded after three months of the plantation was 87 cm in T4 treatment. The increase in plant height was not statistically significant among all treatments ($P = 0.4004$). No visible stress on leave color and leaf size was observed in all treatments along with the control.

Gerbera jamesonii is a commercially important herbaceous perennial plant. Gerbera was the smallest plant grown in the study. Plant height in different substrates was statistically different among control vs. all treatments and T2 vs. T5, T6 ($P < 0.0001$). The maximum height of *Gerbera jamesonii* was recorded in control T1 followed by T2. Comparatively less height was observed in other treatment combinations. Nutrient availability is greatly affected by the initial pH and electrical conductivity of the substrate. The pH range of 5.0-7.2 is considered suitable for gerbera. An increase in pH of the substrate during the study resulted in poor growth of the plant.

Lilium wallichianum plants are best grown in a greenhouse and blooms in winters and withers away as summer approaches. In Lilium the maximum plant height was observed in T3 and T6. Difference observed in plant height was statistically significant in T1 vs. T3 and T1 vs. T6 (P = 0.0035). No significant different was observed in plant height in rest all treatment combinations. Plant height recorded in other treatments containing rice straw and sugarcane bagasse was better than the control (T1). Difference observed in other physical growth parameters of *Lilium wallichianum* were also non significant indicating incorporation of residues had a positive impact on overall plant growth.

Tagetes erecta is a popular ornamental plant in the region that is extensively used in cultural festivals and decorations. The increase in plant height was statistically significant among T1 vs. T4, T2 vs. T4 and T4 vs. T5 (P < 0.0001). Maximum plant height of *Tagetes erecta* was observed in T1 and T2, followed by T5 and T6. *Tagetes erecta* usually grow better in well-drained soil and a pH range of 6.5-7.5. Waterlogged soil and an increase in pH were not found suitable for the growth of *Tagetes erecta*. Although the plants managed to survive in all treatments but growth of physical parameters was better in soil and biochar. The incorporation of residues in media was not found to promote the growth of *Tagetes erecta*.

5.1.4.2. Number of leaves

The number of leaves of the ornamental plants grown in six treatments during the study is provided in Table 5.5 in descending order.

Table 5.5 Number of leaves indicating health and yield efficiency of the plant

Plant Species	**Number of Leaves**
Canna indica	T4 (7)>T1, T2 (6)> T3, T5 (5)>, T6 (4)
Gerbera jamesonii	T1 (8)> T2, T5, T3 (5)> T4, T6 (4)
Lilium wallichianum	T1 (53)> T4, T5, T6 (47), T3 (44), T2 (34)
Tagetes erecta	T2 (47)> T1 (27)> T3, T6 (25)> T4 (21)> T5 (19)

In *Canna indica* no significant difference in the number of leaves was observed for all treatments indicating good adaptation of the plant to different substrates

(P = 0.7596). In *Gerbera jamesonii***,** number of leaves in different substrates was statistically different among control vs. all treatments (P < 0.0001). Maximum number of leaves was observed in the control (T1). In all other treatments new leaves were observed but with time leaves size reduced and leaf color was changed from green to yellow. In *Lilium wallichianum*, difference in number of leaves was statistically significant among T1 vs. T2, T1 vs. T6 and T2 vs. T4, T5 and T6 (P = < 0.0001). Maximum number of leaves was observed in control (T1) and the minimum was observed in T2. No significant difference in number of leaves was observed among treatments T3, T4, T5, and T6. In *Tagetes erecta*, number of leaves in T2 was significantly higher, almost double the number of leaves in other treatment combinations (P = < 0.0001). No significant difference in the number of leaves was observed among other treatments i.e. T1, T3, T4, T5, and T6. Riaz at el. (2015) reported that increase in number of leaves can be attributed to adequate availability of nutrients like nitrogen in the growing substrate. Good number of leaves reflect suitability of the plants to its environment and the growth media.

5.1.4.3. Soil Plant Analysis Development chlorophyll (SPAD) units

Green leaves are fundamental for various functioning of the plants like photosynthesis, gaseous exchange, transpiration, etc. The colour of leaves can be used to identify the stress level of the plant due to its adaptation to environmental changes. Estimation of the chlorophyll content of the leaves is often used to predict the physiological condition of the leaves (Yuan et al., 2016). In the present study handheld, Soil Plant Analysis Development chlorophyll (SPAD) meter was used to get SPAD units which are proportional to the amount of chlorophyll present in the leaves.

In *Canna indica* maximum SPAD reading was observed in T4 and T5. Except for T6, all treatments were having SPAD reading higher than the control T1 indicating good leave health of the canna plants in all treatments. Many studies have reported the potential of canna species in the treatment of wastewater due to its much better growth when irrigated with wastewater. In *Gerbera jamesonii*, maximum SPAD reading was observed in T1 followed by T2 indicating adaption of the plant to soil and biochar, rest of the treatments had lower SPAD readings. Under wastewater stress, the size of the gerbera leaves became smaller and yellowing of leaves was also observed. An increase in pH of the substrate after the addition of wastewater may have caused chlorosis of the plant which resulted in the yellowing of the leaves.

In *Lilium wallichianum,* maximum SPAD reading was observed in T5 followed by control (T1). No significant difference in SPAD units in other treatments was observed. In *Tagetes erecta*, maximum SPAD units were observed in T1 and T2, rest of the treatments had lower SAPD units indicating stress condition. Browning of leaves edges was observed in *Tagetes erecta* plants in treatment combinations which may be attributed to manganese or iron toxicity. Increased absorption of iron and manganese in the *Tagetes erecta* plant leaves was also observed in the elemental analysis.

5.1.4.4. Flowering

The number of flowers produced in each plant and the days to flower emergence are provided in Table 5.6.

Table 5.6 Number of flowers produced and days to flower emergence showing efficacy of the substrate materials for plant growth

Plant	Flowering	T1	T2	T3	T4	T5	T6
Canna indica	Number of flowers	7	5	4	7	5	6
	Days to flower emergence	105	106	103	99	103	105
Gerbera jamesonii	Number of flowers	4	0	0	0	0	0
	Days to flower emergence	61	_	_	_	_	_
Lilium wallichianum	Number of flowers	7	5	7	5	6	7
	Days to flower emergence	33	33	43	38	32	45
Tagetes erecta	Number of flowers	11	16	4	1	2	2
	Days to flower emergence	45	38	64	69	72	55

In *Canna indica,* budding was initiated in the month of May and the first flowering was observed in T4 after 99 days which was statistically at par with other treatments where first flowering was observed after 103 to 106 days. Towards the end of May flowering was observed in all treatment combinations. Flowering was observed in all canna plants till the end of the study i.e. June. Maximum numbers of flowers were observed in T4 and T1 i.e. 7 each. No significant difference in the number of days for the emergence of the first flower was observed in all treatments.

Gerbera jamesonii was found to grow best in control (T1). Budding and flowering were observed only in control in the month of April where it produced one flower in two pots and two flowers in the one pot. Total 4 flowers were produced having an average stalk length of 47.42 ± 7.9 cm and flower diameter of 9.1 ± 0.66 cm. Poor growth of gerbera was observed in all other treatments. No flowering was observed in any other treatment combination indicating the prepared treatments were not suitable for flowering in gerbera.

In *Lilium wallichianum*, budding was first observed within 3 weeks of plantation in all treatment combinations. Flowering was first observed in March end in T5 (32 days) followed by T1, T2 (33 days each) and T4 (38 days). The maximum time for flowering was observed in T3 and T6 i.e. after 43 and 45 days respectively. The average flower diameter observed in various combinations was 4.7 cm in T1, 4.8 cm in T2, 5 cm in T3, 4.6 cm in T4, 5.2 cm in T5, and 5 cm in T6. Stalk length observed was 5.8 cm in T1, 6 cm in T2, T3, and T6, 5.2 cm in T4, and 7.6 cm in T5. Maximum numbers of flowers were observed in T1, T3, and T6 i.e. 7 each. On an average 2-3 flowers were produced in each plant. Production of good quality flowers indicates adequate availability of nutrients in the substrate. Organic matter and nitrogen (N), phosphorus (P) and potassium (K) content in the substrate have positive relationship with flowering indices (Riaz et al. 2015).

In *Tagetes erecta*, budding and flowering were initiated in the month of March. Maximum numbers of flowers were produced in T1 and T2 i.e. 11 and 16 respectively. Comparatively less flowering was observed in T3 (4 flowers), T5 and T6 (2 flowers each). The plant grown in T2 took minimum number of days for first flower (38 days) followed by T1 (45 days) and T6 (55 days). First flower was observed after 72 days in T5. *Tagetes erecta* managed to survive in the prepared treatments but failed to produce a good number of flowers.

5.1.4.5. Stem thickness

In *Canna indica*, maximum stem diameter was recorded in T2 and T6 i.e. 2.5 cm and 2.2 cm respectively. The stem diameter in T1 and T3 was 2 cm and in T4 it was 1.8 cm. In *Gerbera jamesonii*, the stem diameter observed was in the range of 0.4 cm (T3, T6) to 0.5 cm (T1, T2, and T4). In *Lilium wallichianum*, no significant difference in stem diameter was observed in all treatments. The maximum stem diameter observed was 1.14 cm (in T2), and 1 cm (in T1, T3, T4, T5, and T6). In *Tagetes erecta*, stem diameter observed were 0.9 cm in T2, 0.8 cm in T1 and T5, 0.7 cm in T6, and 0.6 cm in T3 and T4.

Fig. 5.3: Flowering observed in (a) *Tagetes erecta*, (b) *Gerbera jamesonii*, (c) *Lilium wallichianum*, (d) *Canna indica*

Location- Lovely Professional University (LPU) Greenhouse, Punjab, India

(latitude 31.2560° N, longitude 75.7051° E, altitude 234 m)

5.1.4.6. Microscopic examination of plant parts

Root and stem cross-section of the plants were observed under the microscope to check the formation of the xylem, phloem and spaces. The formation of cells filled with gas spaces in roots and shoots is known to facilitate the diffusion of water, gases, minerals, and nutrients. Leaf epidermis peels were also observed microscopically to check stomata density.

In *Canna indica* and *Lilium wallichianum*, leaf anatomy revealed no significant variation among the plants grown in six treatments. Good density of stomata was visualized in all treatments indicating good adaption of both plants in the prepared substrates and waterlogged conditions. Fig. 5.4 and Fig. 5.5 show the microscopic images of leaf peel of *Canna indica* and *Lilium wallichianum* grown in six treatments. In *Gerbera jamesonii* and *Tagetes erecta*, stem cross-section showed the formation of xylem, phloem, and spaces only in T1 and T2. In the rest of the treatments, tissue structure was not well developed indicating less tolerance of the plants in the prepared substrates and waterlogged conditions. Fig. 5.6 shows images of the cross-section of the stem of *Tagetes erecta*, *Gerbera jamesonii*, and *Lilium wallichianum*.

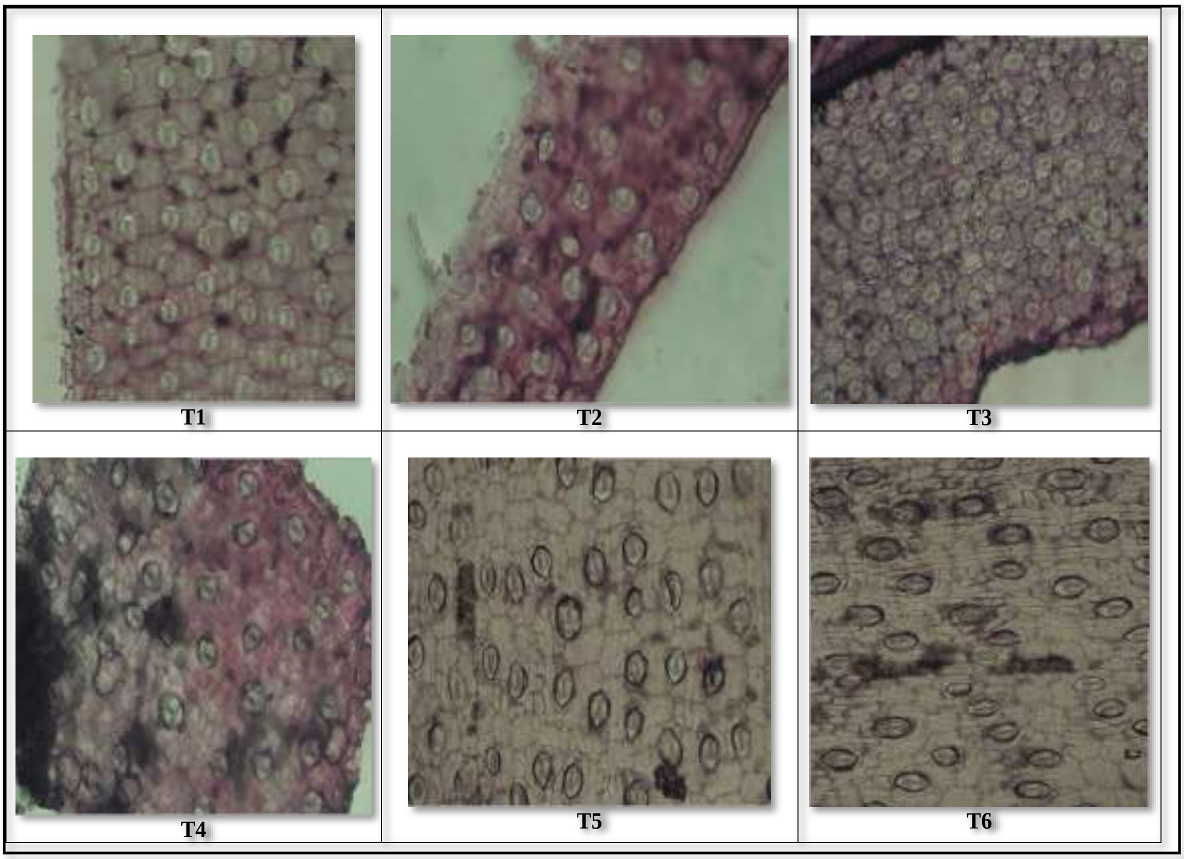


Fig.5.4 Microscopic images of leaf peel of *Canna indica* in six treatments showing stomata which plays an important role in gaseous exchange and photosynthesis (10X magnification)

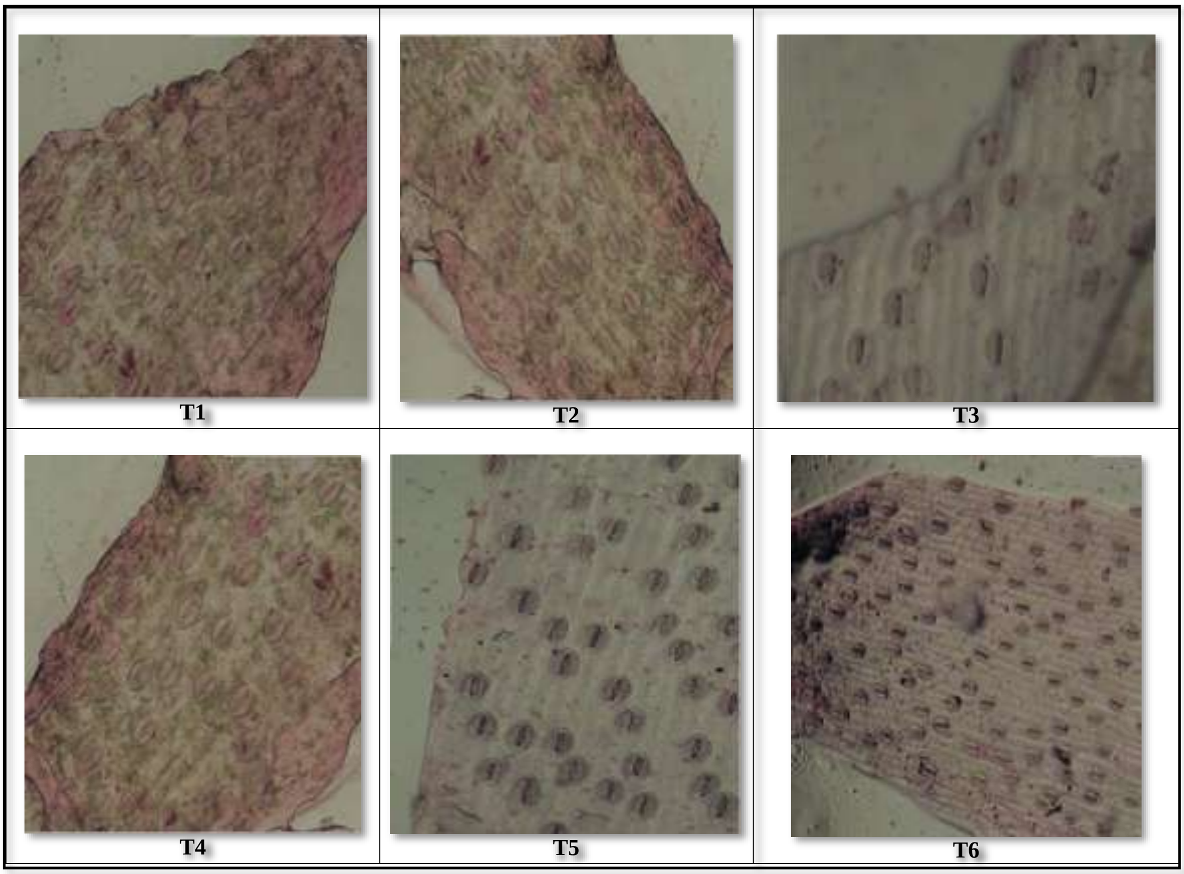


Fig. 5.5 Microscopic images of leaf peel of *Lilium wallichianum* in six treatments showing stomata which plays an important role in gaseous exchange and photosynthesis (10X magnification)

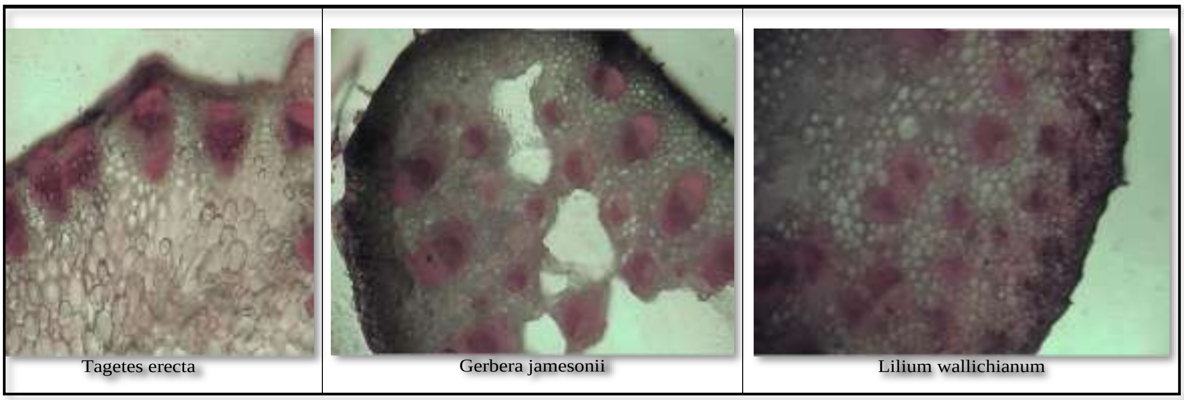


Fig. 5.6 Microscopic images of the cross-section of the stem of *Tagetes erecta*, *Gerbera jamesonii magnification*, and *Lilium wallichianum* showing tissues that facilitate conduction of water and minerals

(4X magnification)

5.1.4.7. Root morphology and biomass

Roots of the plants harvested at the end of the study were observed for morphology and biomass. Fig. 5.7 shows the roots of various plants harvested at the end of the study. Details of shoot length, root length and plant biomass are provided in Table 5.7. In *Canna indica*, increase in plant biomass in all treatments was observed which may be correlated with the utilization of nutrients present in wastewater for plant growth. The observation was consistent with the previous reports of high nutrient removal from wastewater using canna based constructed wetlands (Haritash et al., 2015)**.** Canna plants had big well developed roots in all treatments. In addition to providing anchorage, absorption of water, and nutrient uptake, plant roots are known to provide surface area for various microbial communities that help in the degradation of pollutants from wastewater. Well-developed root systems and diverse microflora are advantageous in phytoremediation (Chandanshive et al., 2018).

In *Gerbera jamesonii* plants had fibrous roots with fine root hairs. Root systems were well developed in T1 and T2. All plants were having some thick roots and numerous thin roots. T1 was having 22 thick roots followed by T2 having 16 thick roots each. T3, T5, and T6 were having around 9-10 thick roots whereas T4 was having 8 thick roots. Maximum shoot (27.4 ± 0.7 cm) and root length (17.7 ± 2.5 cm) were observed in control (T1). T5 was also observed to have a good shoot length (21.9 ± 5.3 cm) and root length (15.2 ± 2.5). The dry weight of the plants was in order T1>T2>T5>T3>T6>T4.

In *Lilium wallichianum*, well-developed root system was observed in T5 with a root length of 25.7 ± 9.3 cm and root spread of 8.0 ± 4.4 cm. Maximum numbers of basal bulbs were also observed in T5. The number of bulbs present in the plants was 8 ± 1 in T1, 7.3 ± 0.5 in T2, 10.3 ± 1.5 in T3, 6.3 ± 1.1 in T4, 10.3 ± 5.0 in T5, and 6.6 ± 2.5 in T6. No significant difference in root length was observed in other treatments. Biomass was also maximum in T5 followed by T4> T1> T3> T2> T6. *Tagetes erecta*, plants were having fine fibrous roots which were well spread throughout the substrate. Good root lengths were observed in T3 and T5 i.e. 26.1 ± 8.2 cm and 25.3 ± 2.5 cm respectively. No significant difference in root length in other treatments was observed. Maximum shoot length and biomass were observed in T1 and T2.

Table 5.7 Shoot length, root length and dry mass of *Gerbera jamesonii*, *Lilium wallichianum*, and *Tagetes erecta* grown in six treatments. Values are given as the mean ± S.D

Plant	Treatment	Shoot Length (cm)	Root Length (cm)	Root side spread (cm)	Dry weight (gm)
Gerbera jamesonii	T1	27.4 ± 0.7	17.7 ± 2.5	7.7 ± 1.2	3.4 ± 1.0
	T2	24.9 ± 6.2	11.8 ± 3.8	5.9 ± 1.9	2.2 ± 0.8
	T3	19.1 ± 1.0	11.8 ± 3.2	5.6 ± 0.6	1.3 ± 0.3
	T4	12.6 ± 2.5	10.5 ± 1.9	5.2 ± 0.9	1.0 ± 0.1
	T5	21.9 ± 5.3	15.2 ± 2.5	5.9 ± 1.9	2.1 ± 1.2
	T6	12.6 ± 2.5	9.6 ± 0.7	5.4 ± 0.7	1.2 ± 0.2
Lilium wallichianum	T1	38.0 ± 2.5	8.5 ± 0.4	3.7 ± 1.2	3.2 ± 0.4
	T2	43.4 ± 4.4	10.5 ± 0.7	3.7 ± 1.2	2.8 ± 0.4
	T3	50.7 ± 4.3	13.5 ± 2.9	6.7 ± 2.9	3.0 ± 0.7
	T4	45.3 ± 4.7	10.9 ± 3.6	3.7 ± 0.1	3.2 ± 0.7
	T5	46.1 ± 8.5	25.7 ± 9.3	8.0 ± 4.4	4 ± 0.7
	T6	46.9 ± 5.5	10.5 ± 1.9	3.3 ± 1.4	1.7 ± 0.4
Tagetes erecta	T1	66 ± 2.5	17.7 ± 2.5	16.4 ± 1.2	6.1 ± 0.3
	T2	75.5 ± 2.7	15.2 ± 2.5	12.5 ± 3.4	3.2 ± 1.2
	T3	47.4 ± 8.1	26.1 ± 8.2	4.8 ± 4.5	2.8 ± 2.9
	T4	35.5 ± 2.1	16.5 ± 7.7	5.8 ± 3.8	1.1 ± 0.1
	T5	57.9 ± 5.9	25.3 ± 2.5	8 ± 1.9	2.6 ± 1.1
	T6	45.8 ± 8.7	13.0 ± 2.6	5.0 ± 1.2	1.7 ± 1.0

Fig. 5.7 Root biomass of the ornamental plants harvested at the end of the study (a) *Tagetes erecta*, (b) *Canna indica*, (c) *Lilium wallichianum*, and (d) *Gerbera jamesonii.* Rich root biomass indicates more surface area for microbial growth and efficient nutrient uptake

5.1.4.8. Elemental analysis

Lilium wallichianum and *Canna indica* were screened out of the four ornamental plants studied on the basis of their growth parameters for elemental analysis. Quantity of the elements analyzed in the leaf samples of both the plants is presented in Table 5.8. Analysis of leaves of *Lilium wallichianum* showed no significant difference in uptake of nitrogen (P=0.3382), sulphur (P=0.0774), and potassium (P=0.2594) in treatments containing agricultural residues versus control, however, a significant uptake of phosphorus (P=0.0363) and calcium (P=0.001) was observed in treatments containing sugarcane bagasse. In *Canna indica* leaves, increased nitrogen (P<0.001) and phosphorus uptake (P<0.001) was observed in plants grown in treatment containing rice straw residues. Also, increased potassium (P=0.004) and calcium uptake (P=0.0331) was observed in plants grown in treatment containing agricultural residues.

Table 5.8 Quantity of the elements analyzed in leaf samples of *Lilium wallichianum*, and *Canna indica*, grown in control and in treatments containing rice straw residue and sugarcane bagasse. Quantity of elements are expressed in ppm. Values are given as the mean± S.D

Particular of Elements	Concentraton of Required Elements (ppm)	*Lilium wallichianum*			*Canna indica*		
		Control	Treatment containing rice straw residue	Treatment containing sugarcane bagasse	Control	Treatment containing rice straw residue	Treatment containing sugarcane bagasse
Nitrogen	20000-50000	32205.67 ± 2286.92	30807.67 ± 2555.43	29416 ± 1284.51	23800 ± 1034.38	29410.33 ± 1515.60	16115.67 ± 1052.19
Phosphorus	2000-5000	1976 ± 268.42	2481.67 ± 179.72	2504 ± 167.44	2031 ± 97.89	2630.67 ± 55.08	1861.33 ± 102.93
Sulphur	1000-3000	1823 ± 150.34	2098 ± 198.27	1796 ± 17.09	2277.67 ± 103.64	2191 ± 229.24	1586.33 ± 87.03
Potassium	10000-50000	28450.33 ± 1347.73	28350.67 ± 857.33	27081.33 ± 724.59	38338 ± 1728.62	55284 ± 3592.80	51470 ± 1870.86
Calcium	1000-10000	17509.67 ± 1839.57	12630 ± 862.03	26010 ± 1639.38	5847.67 ± 140.46	7433 ± 187.22	22050 ± 1599.41
Magnesium	1000-4000	5434 ± 1137.21	3019.67 ± 104.01	4067.33 ± 108.62	5178 ± 225.81	5442.33 ± 214.85	3464 ± 126.87

5.1.5. Substrate for the constructed wetland

Utilizing agricultural wastes as substrates in constructed wetlands can offer a number of benefits. The addition of organic matter like rice straw, sugarcane bagasse, and biochar as supplementary materials improves substrate properties by providing more carbon, increasing nutrient absorption, and promoting the growth of microorganisms (Ji et al., 2022). The addition of agricultural residue has been shown to increase total organic and dissolved organic carbon in the soil and improve soil health. It is further shown to promote microbial growth by providing a large surface area for microbes to function and grow. It is likely that microorganisms present in the constructed wetland system can utilize organic agricultural waste as the carbon source to produce valuable molecules like amino acids, organic acids, and enzymes. Over time it will favour the growth of diverse microbial populations in the constructed wetland.

An issue with conventional wastewater treatment plants is the generation of secondary pollutants like sludge, which requires further processing and disposal (Stefanakis, 2019). Interestingly, constructed wetlands do not generate any further pollutants, in fact, the harvested biomass and the saturated substrates generated from the wetland units can be further converted into other high-value products. The harvested biomass can also be further utilized as feedstock for biorefining or as a raw material for the extraction of natural polymers, preparation of catalytic materials, and carbon based adsorbents (Maroušek and Maroušková, 2021; Zhou and Wang, 2020). Results of the present study demonstrate that incorporation of agricultural residues like rice husk and sugarcane bagasse improve substrates properties and using ornamental plants provide revenue generation opportunity.

5.1.6. Ornamental plants for constructed wetland

Differences were observed in the growth parameters of the selected plants which indicate differential tolerance to the wastewater. It was observed that the adaptability of *Canna indica* and *Lilium wallichianum* was better in the designed system under wastewater stress conditions. *Canna indica* plant species have been explored in a number of wetland studies (Nakase et al., 2019; Zamora et al., 2019; Calheiros et al., f2015; Pinninti et al,. 2022; Nema et al., 2020) and are reported to be an efficient plant species that can survive the wastewater stress and improve the treatment efficiency of the system. Apart from its high growth rate, *Canna indica* plants are also known to have superior tolerance and ability to survive in water-logged conditions. Within three months of the plantation, *Canna indica* plants developed into dense strands and produced flowers in all the media. The nutrients present in the wastewater were utilized by the plants for their growth which was evident from the increase in height, leaves, and flowering. Haritash et al. (2015) also reported high biomass production in a *Canna*-based treatment system that is directly related to the uptake of nutrients from wastewater and media.

In the initial 2-3 weeks of plantation of *Lilium wallichianum*, browning was observed on the tips of leaves which were replaced with healthy leaves in three weeks. In *Tagetes erect*a, the browning of leaves was observed throughout the study period. Despite this, new branches developed in the medium, and flowers were also produced. However, the number of flowers produced was less. Flowers of *Lilium wallichianum* appeared two months after plantation followed by *Tagetes erecta* and *Canna indica* that appeared after three months and four months of plantation respectively. Each *Lilium wallichianum* plant produced two to three flowers per plant in each mesocosm. *Tagetes erecta* produced six to seven flowers per plant in M1, only two to four flowers per plant in M2, and four to five flowers per plant in M3.

In the present study, *Canna indica* and *Lilium wallichianum* were able to tolerate the wastewater stress whereas *Tagetes erecta* showed browning of leaves edges in all media combinations. No signs of stress were observed in *Canna indica* plants grown in any media which is similar to the study conducted using *Canna lily* species by Haritash et al. (2015) for the treatment of domestic wastewater. No direct wastewater stress could be established in *Lilium wallichianum* as well, which shows the ability of these two plants to survive and produce flowers in the wetland system.

Gerbera jamesonii failed to show good growth in terms of plant height and number of leaves in any treatment combinations. In gerbera, yellowing of leaves and loss of green pigments were observed under wastewater-irrigated conditions in all treatments with lower chlorophyll content. No flowering was observed in any treatment except in the control indicating that treatment combinations were not found to be suitable for the growth of the gerbera plant.

Tagetes erecta was able to grow well in all treatments but the best growth was observed in the control indicating that the plant was stressed in the wastewater treatments compared to the control. *Tagetes erecta* plants were able to produce flowers in all treatments, but the number of flowers produced in various treatments was less in comparison to the control. Level of iron and magnesium were above the optimum level in all treatments which may have caused the leaves of *Tagetes erecta* to turn brown and speckle. Presence of iron in the coagulation sludge mixed with biowaste has been reported to hamper the availability of phosphorus from the soil to the plants, by turning phosphorus to iron phosphates (Maroušek et al., 2022). An increase or decrease in the pH other than the optimum range can adversely affect plant growth by decreasing nutrient availability and damaging roots. Change in the pH of the substrate affects the solubility of the nutrients which have a direct impact on the growth and development of the plant.

All plant growth parameters were compared to select the best matrix combination for each plant. In *Canna indica*, maximum plant height, number of leaves, and highest chlorophyll content was observed in T4 treatment. Plants grown in T4 also had the maximum number of flowers with the least days to flower emergence. In *Lilium wallichianum*, plants grown in T5 had maximum chlorophyll content, least days to flower emergence, maximum root biomass, and highest plant dry weight. For *Gerbera jamesonii,* no treatment combination was found to be suitable except the control (T1) which had maximum chlorophyll content, stem diameter, shoot length, root length, and plant dry weight. Also, flowering was only observed in T1. In *Tagetes erecta*, plants grown in T2 were observed to have maximum plant height, number of leaves, stem diameter, and chlorophyll content. Plants in T2 also had the maximum number of flowers with the least days to flower emergence.

Overall the treatment combination having 15% rice straw (T4) was found most suitable for the growth of *Canna indica,* while *Lilium wallichianum* was found to grow best in treatment having 25% sugarcane bagasse (T5). *Tagetes erecta* was able to survive in all treatments, however, maximum growth was observed in the treatment having soil amended with only 5% biochar (T2). *Canna indica* produced a maximum of 7 flowers in two treatments (T1, T4), *Lilium wallichianum* produced a maximum of 7 flowers in three treatments (T1, T3, T6), and *Tagetes erecta* produced a maximum of 16 flowers in the T2 treatment. Although *Tagetes erecta* plants flowered in all treatments but the number of flowers produced was less than the control. *Gerbera jamesonii* was not able to survive in any treatment except control where it produced 4 flowers.

5.2. Performance analysis of mesocosm-constructed wetland

In the second part of the study, vertical sub-surface flow mesocosm constructed wetland systems having *Canna indica, Lilium wallichianum*, and *Tagetes erecta* grown in media namely soil and biochar; soil, biochar, and rice straw; soil, biochar, and sugarcane bagasse were evaluated for its wastewater treatment potential. The influent and effluent from mesocosms planted with P1 (*Canna indica*), P2 (*Lilium wallichianum*), P3 (*Tagetes erecta*) in media M1 (Gravel, Sand, Soil, Biochar), M2 (Gravel, Sand, Soil, Biochar, Rice Straw) and M3 (Gravel, Sand, Soil, Biochar, Sugarcane Bagasse) were analyzed for pH, TDS, dissolved oxygen demand (BOD and

COD) and nutrients (PO_4-P, NH_4-N, and NO_3-N) using standard methods for five weeks.

5.2.1. Wastewater characterization

Physiochemical characterization of the influent is shown in Table 5.9. The pH and TDS of the influent varied from 7.12 to 8.2 units and 515 mg/L to 638 mg/L respectively. BOD was found to vary from 84.26 mg/L to 154.17 mg/L with an average value of 123.34 mg/L which is comparable to those reported by Singh et al. (2022) who worked with the same influent. COD values ranged from 157.08 mg/L to 264.18 mg/L with an average value of 215 mg/L.

Table 5.9 Physiochemical characterization of wastewater used in the study (n=15)

Parameter (mg/l*)	Value (average± SD)	Minimum	Maximum
pH	7.65±0.35	7.12	8.2
TDS	582.06±38.09	515	638
Biochemical Oxygen Demand	123.34±24.21	84.26	154.17
Chemical Oxygen Demand	214.86±32.14	157.08	264.18
Ammonia	35.95±6.91	25.68	48.76
Phosphorus	6.58±1.23	4.68	8.43
Nitrate	12.34±3.80	6.12	18.3

* Except for pH

5.2.2. Influence of media on the treatment performance

In the present study rice straw residues were incorporated as media components in M2. In recent years, rice straw is finding increased applications as a solid carbon source in constructed wetlands owing to its high efficiency and low cost. Zhang et al. (2019) reported an average increase of 10.7% in the potential denitrification rate in a combined system of rice straw ponds and surface flow CWs for swine wastewater treatment. The study also reported enhanced gene abundance of 16S rRNA including ammonia-oxidizing bacteria, nitrous oxide reductase, and nitrate reductase, etc. in the CWs. The presence of diverse bacterial population contributes to the pollutant removal efficiency of the wetland (Feng et al., 2021).

Media used in the system is not only a matrix for the physical processes but is also important for plant growth as well as for microbial populations that catalyze various chemical reactions thereby affecting the overall treatment performance of the constructed wetland (Feng et al., 2021). These microbial transformations taking place in the media are the main removal mechanism for the diverse range of pollutants present in the wastewater. Interaction and cooperation among these physical and microbial processes in the constructed wetland system account for the removal of BOD and COD from the wastewater (Ghosh and Gopal, 2010).

Compared to the mesocosms containing sugarcane bagasse, one shortcoming of incorporating rice straw residues was the darker unpleasant colour of the effluent. In the present study, the effluent from the mesocosm containing rice straw residue was observed to be dark brown in colour in comparison to the clear effluent observed from other mesocosms (Fig. 5.8). A similar dark brown colour of the effluents from the rice straw organic channel barriers investigated for their nutrient removal capacity was reported by Liu et al. (2015). Release of the excessive organic carbon from the rice straw causes the unpleasant colour of effluent (Zhang et al., 2019). In organic channel barriers composed of rice straw, Liu et al. (2015) observed the release of carbon during the first three weeks. A similar higher release of carbon was reflected in the brown colour of the effluent from M2 media. Dark- coloured effluent was observed in the initial two-three weeks which is expected to stabilize with the increase in the operation duration. Nonetheless, colour -producing organics can be effectively removed by passing the effluents through another horizontal flow constructed wetland using sand as the main media, as efficiently demonstrated by Saeed and Sun (2013). Anaerobic conditions inside the media are known to accelerate biological decolonization.

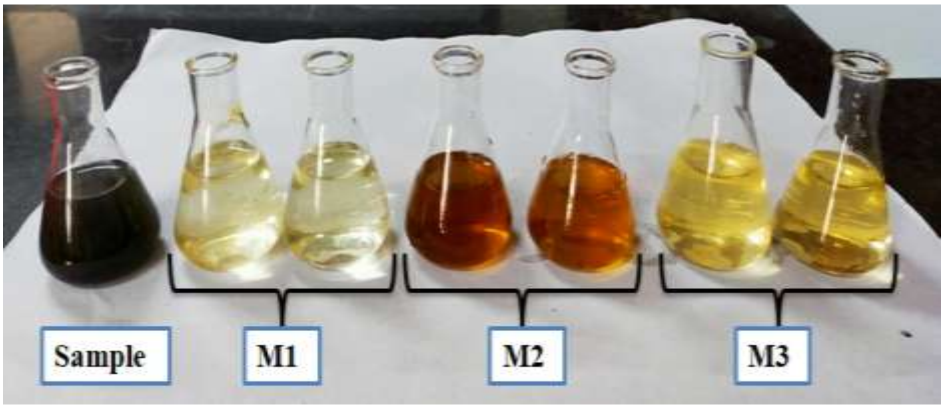


Fig. 5.8: Influent wastewater sample and treated effluent collected from different mesocosms

5.2.3. Influent and effluent analysis for pollutant removal

5.2.3.1. pH and dissolved solids

In the present study, the pH value of the influent varied from 7.62 to 7.81 with an average of 7.73±0.07 units. In mesocosms planted with *Canna indica* (P1), the pH of the effluent was 7.69±0.09 in M1, 7.63±0.10 in M2, and 7.69±0.11 in M3. In systems planted with *Lilium wallichianum* (P2), pH was 7.72±0.07 in M1, 7.67±0.09 in M2, and 7.67±0.08 in M3 whereas, in mesocosms planted with *Tagetes erecta* (P3), pH was 7.72±0.06 in M1, 7.7±0.08 in M2 and 7.65±0.08 in M3 (Table 5.10).

A slight increase in the pH of the effluents was observed which may attribute to the alkaline nature of the biochar (7.9- 8.2) used in the medium. An increase in the pH of the effluent above the permissible water quality standards was also reported by Singh et al. (2022) who explored biochar prepared from pyrolysis of rice husk, as an adsorbent for contaminants. An increase in pH also reflects the presence of NaOH and $Ca(OH)_2$ in water. The increase observed in the pH of all effluents was within the permissible discharge standards for inland surface water i.e. 5.5 to 9.0 given by Central Pollution Control Board (CPCB).

Table 5.10 pH and Total Dissolved Solids (TDS) observed in influent and effluents from mesocosms planted with P1 (*Canna indica*), P2 (*Lilium wallichianum*), P3 (Tagetes erecta) in media M1 (Gravel, Sand, Soil, Biochar), M2 (Gravel, Sand, Soil, Biochar, Rice Straw) and M3 (Gravel, Sand, Soil, Biochar, Sugarcane Bagasse)

Plant		**pH**	**TDS (mg/L)**
	Influent	7.73±0.07	588±19.13
P1	M1	7.69±0.09	531±39.24
	M2	7.63±0.10	580.6±19.74
	M3	7.69±0.11	521.2±17.44
P2	M1	7.72±0.07	553.4±31.93
	M2	7.67±0.09	598.2±24.68
	M3	7.67±0.08	561.4±29.94
P3	M1	7.70±0.08	570.8±54.4
	M2	7.7±0.08	597.8±21.28
	M3	7.65±0.08	556±19.09

The TDS content in the influent was 588±19.13 mg/L. In mesocosms planted with *Canna indica* (P1), the TDS of the effluent was 511±61.56 in M1, 580.6±19.74 in M2, and 481.2±57.65 in M3. In systems planted with *Lilium wallichianum* (P2), TDS was 553.4±31.93 in M1, 598.2±24.68 in M2, and 561.4±29.94 in M3 whereas, in mesocosms planted with *Tagetes erecta* (P3), TDS was 570.8±54.4 in M1, 597.8±21.28 in M2 and 556±19.09 in M3. Table 5.10 shows the average pH and TDS observed in all mesocosms and the concentration in the influent and effluents are presented in Fig. 5.9.

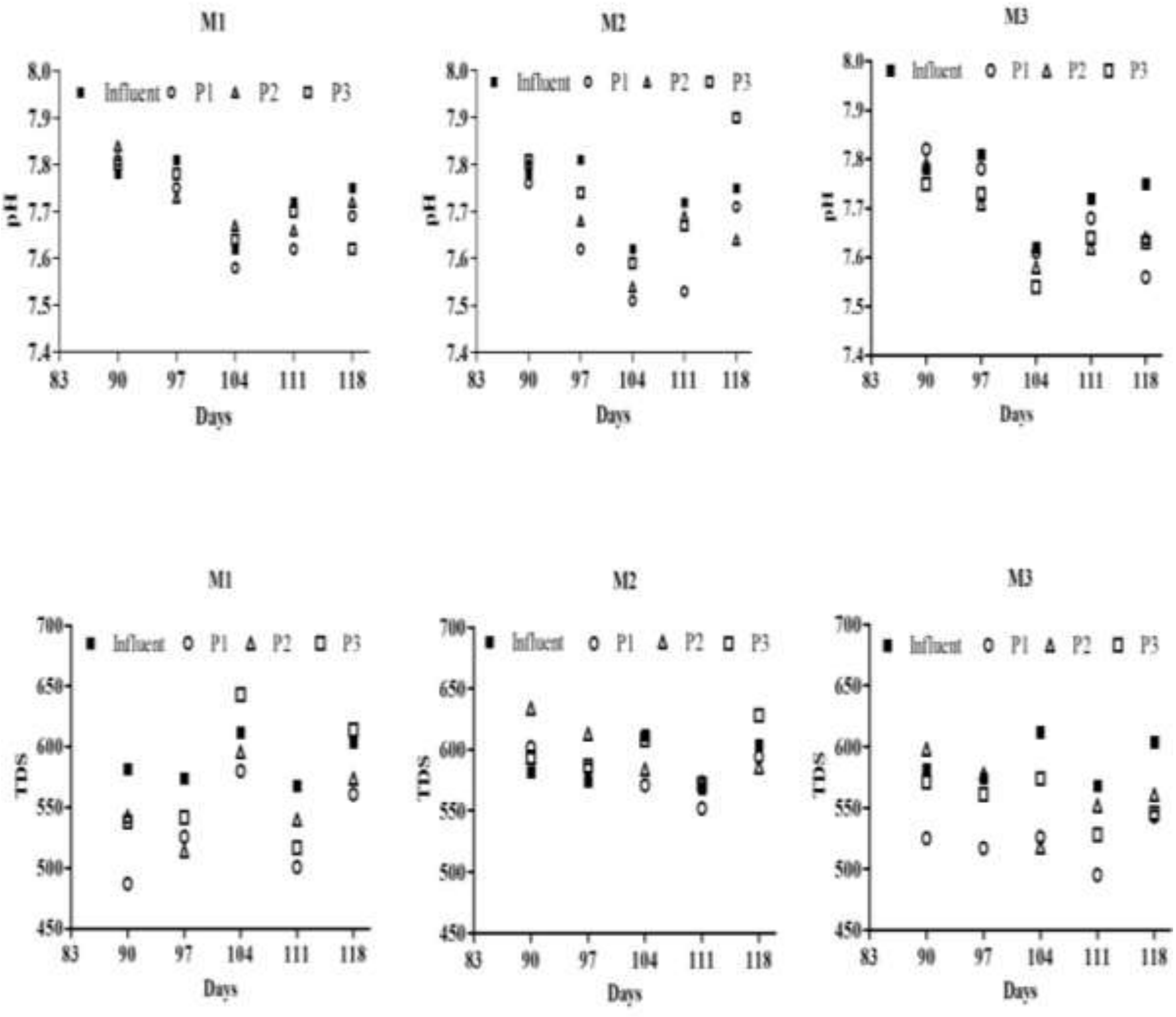


Fig. 5.9. pH and TDS concentration in the influent and effluent from mesocosms planted with P1 (*Canna indica*), P2 (*Lilium wallichianum*), P3 (Tagetes erecta) in media M1 (Gravel, Sand, Soil, Biochar), M2 (Gravel, Sand, Soil, Biochar, Rice Straw) and M3 (Gravel, Sand, Soil, Biochar, Sugarcane Bagasse)

5.2.3.2. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD)

The average values of organics and nutrients in the influent and effluent along with the percentage removal efficiency are presented in Table 5.11. In mesocosms planted with *Canna indica* (P1), BOD effluent concentration was 71.4 mg/L in M1, 58.2 mg/L in M2, and 56.6 mg/L in M3 with an average removal efficiency of 47%, 57%, and 58%, respectively. The COD effluent concentration of 90.2 mg/L in M1, 84.8 mg/L in M2, and 74.2 mg/L in M3 with an average removal efficiency of 56%, 59%, and 64%, respectively was observed. Maximum removal of BOD i.e. 58% and COD i.e. 64% was observed in M3 media which was amended with 5% biochar and 15% sugarcane bagasse.

Table 5.11 Average concentration of organics and nutrients observed in the influent and effluent from all mesocosms planted with P1 (*Canna indica*), P2 (*Lilium wallichianum*), P3 (Tagetes erecta) in media M1 (Gravel, Sand, Soil, Biochar), M2 (Gravel, Sand, Soil, Biochar, Rice Straw) and M3 (Gravel, Sand, Soil, Biochar, Sugarcane Bagasse)

			Effluent					
			Average ± S.D			**Removal Percentage (%)**		
			Media			**Media**		
Plant	**Parameter Evaluated**	**Influent**	**M1**	**M2**	**M3**	**M1**	**M2**	**M3**
P1	BOD	135.4±11.99	71.4	58.2	56.6	47.26	57.01	58.19
	COD	209±23.32	90.2	84.8	74.2	56.84	59.42	64.49
	NH_4-N	33.46±6.50	19.47	17.52	15.79	41.81	47.64	52.81
	NO_3-N	12.07±1.94	7.85	6.73	5.41	34.96	44.24	55.18
	PO_4-P	6.4±1.12	4.92	4.68	4.43	23.13	26.88	30.78
P2	BOD	135.4±11.99	78.2	66.2	59.4	42.24	51.1	56.12
	COD	209±23.32	111.8	97.6	79.4	46.5	53.3	62
	NH_4-N	33.46±6.50	20.46	17.70	15.76	38.85	47.10	52.90
	NO_3-N	12.07±1.94	7.51	6.95	5.56	37.78	42.42	53.94
	PO_4-P	6.4±1.12	5.43	4.83	4.53	15.16	24.53	29.22
P3	BOD	135.4±11.99	82.8	77.8	68.2	38.84	42.54	49.63
	COD	209±23.32	119.2	112.6	99.8	42.96	46.12	52.24
	NH_4-N	33.46±6.50	25.50	24.84	21.72	23.79	25.76	35.09
	NO_3-N	12.07±1.94	9.01	7.62	6.63	25.35	36.87	45.07
	PO_4-P	6.4±1.12	5.63	5.39	5.36	12.03	15.78	16.25

In mesocosms planted with *Lilium wallichianum* (P2), BOD effluent concentration was 78.2 mg/L in M1, 66.2 mg/L in M2, and 59.4 mg/L in M3 with an average removal efficiency of 42%, 51%, and 56%, respectively whereas COD effluent concentration was 111.8 mg/L in M1, 97.6 mg/L in M2 and 79.4 mg/L in M3 with an average removal efficiency of 46%, 53%, and 62%, respectively. Highest removal of BOD i.e. 56% and COD i.e. 62% was observed in M3 media which is similar to the effluent concentrations observed in mesocosms planted with *Canna indica*.

In mesocosms planted with *Tagetes erecta* (P3), BOD effluent concentration was 82.8 mg/L in M1, 77.8 mg/L in M2, and 68.2 mg/L in M3 with an average removal efficiency of 38%, 42%, and 49%, respectively. Observed COD effluent concentration was 119.2 mg/L in M1, 112.6 mg/L in M2, and 99.8 mg/L in M3 with average removal efficiency of 42%, 46%, and 52%, respectively. BOD and COD removal from mesocosms planted with *Tagetes erecta* was on the lower side in comparison to the other two plants. Despite this, the influence of the species of the ornamental plant used on the removal efficiency was non-significant ($p>0.05$). This finding is in agreement with a similar study conducted by Burgos et al. (2017) which evaluated the performance of ornamental plants for sewage treatment under different organic loading.

The concentration of BOD and COD observed in the influent and effluent over the operation period in all media combinations is represented in Fig. 5.10. A removal efficiency of BOD and COD above 40%-50% has been documented with ornamental plants. For instance, Haritash et al. (2015) reported 69.8%-96.4% of BOD and 63.6%-99.1% of COD removal using *Canna lily* plant species in constructed wetland for removal of carbon, nitrogen, and phosphorus from domestic wastewater. Another similar study by Calheiros et al. (2015) for the treatment of wastewater using ornamental plants in constructed wetland, reported above 90% removal efficiency of organics (BOD, COD), up to 99% removal of total coliforms, and up to 84% removal of NH_4^+ and 94% removal of PO_4^{3-}.

The removal efficiency of BOD and COD in the mesocosms investigated in the present study was observed to be around 39%-58% and 43%-65%, respectively which were slighted on the lower side. This might be due to the less retention time of 24 hours used in the present study. A similar study using *T. angustata* planted in a gravel bed for tertiary treatment of secondary effluent from an effluent treatment plant receiving wastewater from a milk-processing plant and domestic sewage from the staff quarters reported an almost 3 folds increase in removal efficiency of BOD and COD when retention time was increased from 1 to 2 days. It further reported a nearly double increase in the removal efficiency with the hydraulic retention time of 4 days (Ghosh and Gopal, 2010).

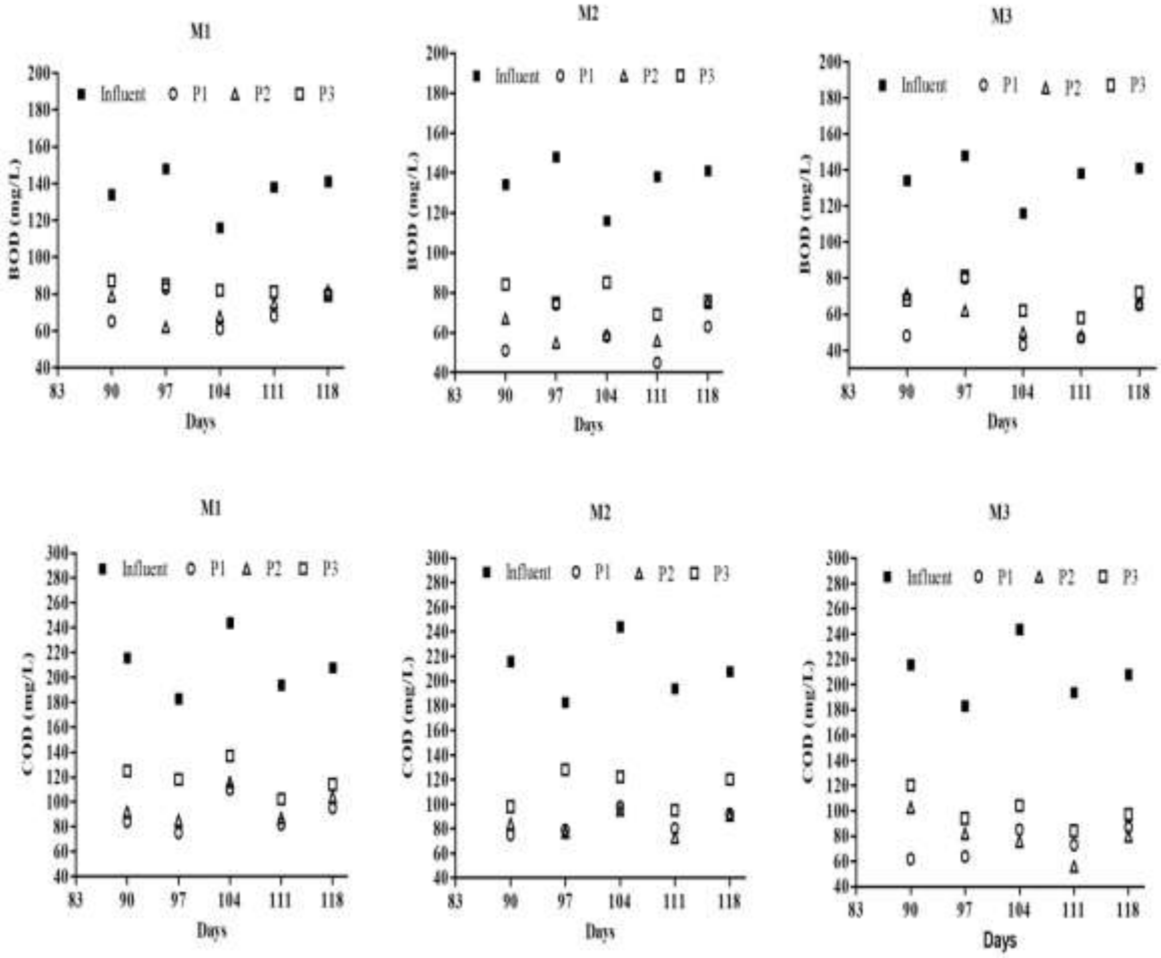


Fig. 5.10. BOD and COD concentration in the influent and effluent from mesocosms planted with P1 (*Canna indica*), P2 (*Lilium wallichianum*), P3 (Tagetes erecta) in media M1 (Gravel, Sand, Soil, Biochar), M2 (Gravel, Sand, Soil, Biochar, Rice Straw) and M3 (Gravel, Sand, Soil, Biochar, Sugarcane Bagasse)

5.2.3.3. Nutrients

The concentration of nutrients (NH_4-N, NO_3-N, and PO_4-P) in the influent and effluent from all mesocosms over the operation period is presented in Fig. 5.11. The average concentration of N-NH_4 in the influent was 33.46 mg/l and in the effluent, it varied from 15.76 mg/l to 25.50 mg/l. In effluent from mesocosm planted with *Canna indica* (P1), N-NH_4 concentration was below 25.67 mg/l in M1, 28.2 mg/l in M2, and 29.14 mg/l in M3. The removal efficiency was observed to be 41.81% in M1, 47.6% in M2 52.81% in M3. N-NH_4 concentration in the effluent from mesocosms planted with *Lilium wallichianum* (P2) and *Tagetes erecta* (P3) was 20.46 mg/l and 25.5 mg/l in M1, 17.7 mg/l, and 24.84 mg/l in M2, 15.76 mg/l and 21.72 mg/l in M3 respectively. The observed NH_4 removal was similar to those reported by other authors (Verma and Suthar, 2018; Nema et al., 2019; Shukla et al., 2021; Zamora et al., 2019; Burgos et al., 2017; Ghosh and Gopal, 2010; Calheiros et al., 2015). The maximum removal efficiency of N-NH_4 i.e. 53% was in mesocosms planted with *Lilium wallichianum* in M3 media whereas the minimum i.e. 24% was observed in mesocosms planted with *Tagetes erecta* in M1. This shows that M3 media has performed better compared to M1 and M2 media for removing N-NH_4 from the wastewater.

Meanwhile, the average N-NO_3 concentration in the influent was 12.07 mg/l and in effluent concentration varied from 5.41 mg/l to 9.01 mg/l. N-NO_3 concentration in mesocosms planted with *Canna indica* (P1) was 7.85 mg/l in M1, 6.73 mg/l in M2 and 5.41 mg/l in M3. In mesocosms having *Lilium wallichianum* (P2) and *Tagetes erecta* (P3) it was 7.51 mg/l and 9.01 mg/l in M1, 6.95 mg/l and 7.62 mg/l in M2, 5.56 mg/l and 6.63 mg/l in M3, respectively. Maximum removal efficiency of 55.18% was observed in mesocosm having *Canna indica* in M3 and a minimum of 25.35% was observed in mesocosm having *Tagetes erecta* in M1. This result is in agreement with Sandoval et al. (2019a), Sandoval-Herazo et al. (2018), Zamora et al. (2019) and Ghosh and Gopal (2010). Non- significant difference ($p>0.05$) in the removal of N-NO_3 was observed among all media combinations used in the present study. Again, M3 media performed better compared to M1 and M2 media for removing NO_3-N from the wastewater.

The average concentration of PO_4-P in the influent was 6.4 mg/l and in the effluent, it varied from 4.43 mg/l to 5.63 mg/l. PO_4-P concentration in *Canna indica* (P1) mesocosms was 4.92 mg/l in M1, 4.68 mg/l in M2 and 4.43 mg/l in M3, whereas in mesocosms with *Lilium wallichianum* (P2) and *Tagetes erecta* (P3) it was 5.43 mg/l and 5.63 mg/l in M1, 4.83 mg/l and 5.39 mg/l in M2, 4.53 mg/l and 5.36 mg/l in M3 respectively. The PO_4-P removal was similar to those reported by Yadav et al. (2018), Sandoval-Herazo et al. (2018) and Ghosh and Gopal (2010). Maximum removal efficiency of 30.78% was observed in mesocosm having *Canna India* in M3 and a minimum of 12% was observed in mesocosm having *Tagetes erecta* in M1 media. Again, M3 media appears to have performed better compared to M1 and M2 media for removing PO_4-P from the wastewater.

Relatively, higher removal efficiency of pollutants was observed in M2 and M3 media in comparison to M1 media which indicates a positive influence of incorporation of organic waste as constructed wetland media. Non-significant ($p>0.05$) difference was observed in the performance of the mesocosms containing rice straw and sugarcane bagasse except for the darker colour observation of the effluent due to the release of carbon from rice straw which required additional treatment. In general, the incorporation of sugarcane bagasse was observed to have a positive impact on the performance of the mesocosm. A limited number of studies have reported the potential of organic waste, especially agricultural by-products, as media for wetland systems. For instance, Saeed and Sun (2013) reported 74%–79% BOD removal and 59%–66% ammonia removal in vertical-flow constructed wetland filled with sugarcane bagasse as the main media. This study clearly demonstrated the efficiency of the sugarcane bagasse media in improving denitrification in a wetland. Owning to its physical structure sugarcane bagasse offers greater porosity and carbon leaching that supports nitrification and denitrification processes along with removal of biodegradable organics in vertical flow constructed wetland. Organic components of the bagasse may have provided more carbon content that supports the growth of heterotrophic biofilm inside the media. The role of microbes in processes like nitrification, denitrification, and other biogeochemical cycles are the main removal mechanisms in the constructed wetland.

Apart from microbial transformations, plants also contribute to nutrient uptake (Moreira and Dias, 2020). Plants require nutrients for their growth and reproduction. Vymazal (2020) reported that plants play a direct role in the removal of nutrients especially in lightly loaded systems. In the present study, *Canna indica* plants produced significantly higher biomass which points towards high water demand and efficient and more nitrogen plant uptake. *Canna indica* plants have 3-5 times more water demand compared to the other wetland vegetation and nutrient removal depends on the water demand and plant uptake. The increased biomass can be correlated with nutrient assimilation from wastewater (Haritash et al., 2015).

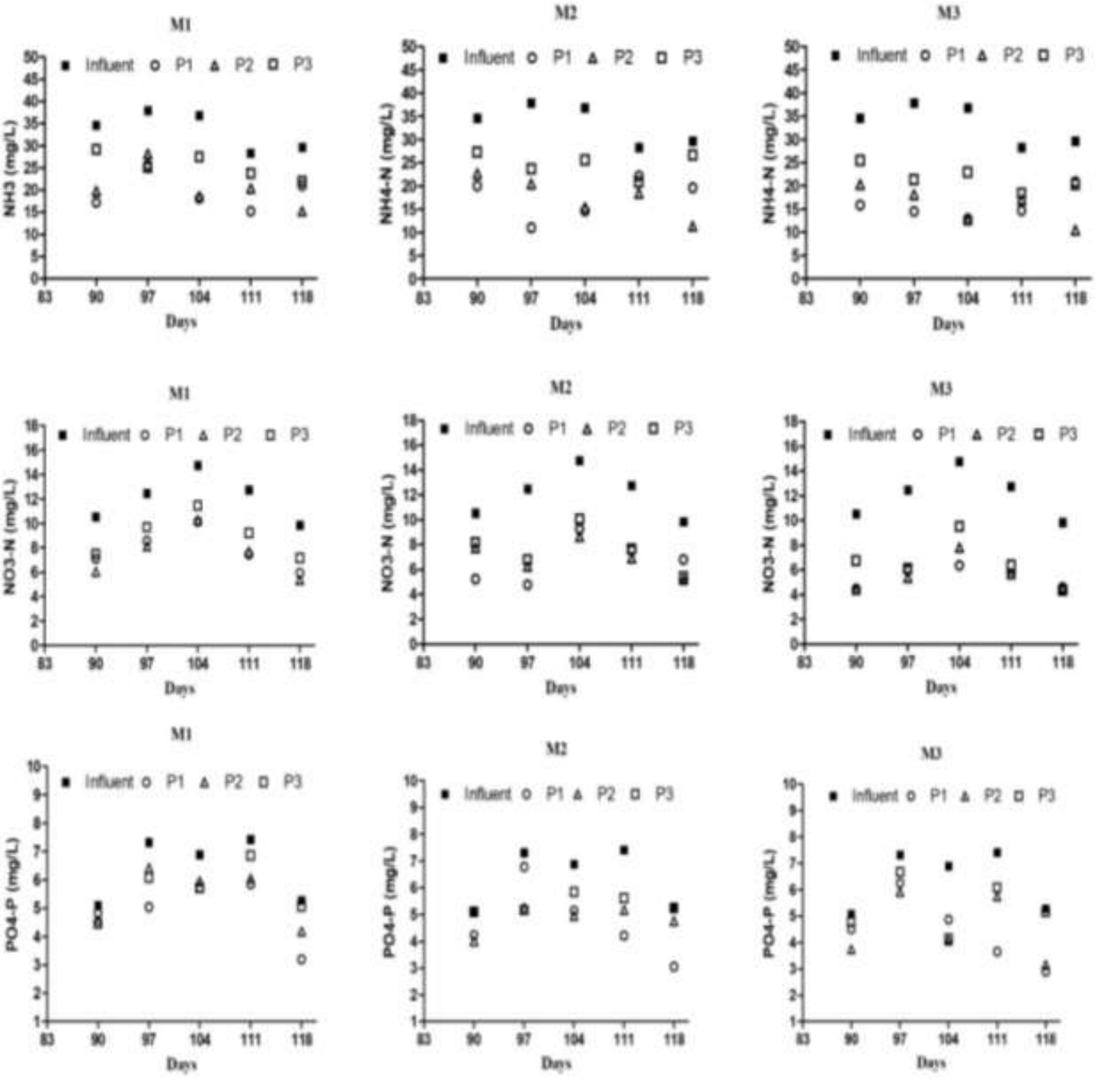


Fig. 5.11 NH_4-N, NO_3-N, and PO_4-P concentration in the influent and effluent from mesocosms planted with P1 (*Canna indica*), P2 (*Lilium wallichianum*), P3 (Tagetes erecta) in media M1 (Gravel, Sand, Soil, Biochar), M2 (Gravel, Sand, Soil, Biochar, Rice Straw) and M3 (Gravel, Sand, Soil, Biochar, Sugarcane Bagasse)

Investigated mesocosms showed an average removal efficiency of 49.21% for BOD, 53.76% for COD, 40.64% for NH_4-N, 41.76% for NO_3-N, and 21.53% for PO_4-P. The result shows that the designed mesocosms are a promising nature-based alternative to the technologically complex and expensive conventional wastewater treatment technologies, with numerous additional ecological benefits. This study also indicates that the locally available organic materials are effective media components for constructed wetlands and after their use in wetlands; these digested organic materials may further be used as an effective source of nutrient-rich fertilizers, or soil amendments in agriculture.

5.3. Design and development of vegetative wetland system

Constructed wetlands have been successfully and efficiently used for several decades as sustainable and economical alternative to the conventional wastewater treatment plants. Recently, there has been a lot of focus on design parameters and operational approaches to enhance effectiveness and long term performance of constructed wetland systems. Well designed system is crucial for effective pollutant removal, to achieve required water quality and to meet the discharge standards. Achieving the planned treatment objectives and associated ecological, social, and economic benefits depends greatly on the design of the wetland system, which also has a substantial impact on treatment performance. Determining and prioritizing the intended purpose of the treatment system is a very important step based on which the system is designed and operated.

5.3.1. Treatment objectives and wastewater characterization

To develop a system for wastewater treatment it's crucial to clearly define the treatment objectives and set clear goals for the treatment process. Wastewater characterization is also necessary to estimate the type and concentration of pollutants. Qualitative and quantitative estimation of influent is important before setting the wetland unit. In the present study, wastewater characterization was done before starting the treatment phase. Type of wastewater influences the choice of the substrate and plants for the system. For heavily polluted wastewater more tolerant plant varieties can be used and for moderately and lightly polluted wastewater, ornamental plant varieties can be installed in the system.

In the present study, removal of organic contaminants was targeted. Wastewater from any source can contain diverse range of pollutants, hence it is necessary to firstly characterize the wastewater for presence of contaminants and accordingly plan the treatment systems. In the present study, vertical flow mesocosm system was used for treatment of wastewater collected from a drain flowing through the University campus. Upstream to the drain was a sewage treatment plant that releases the effluents in the drain. Downstream to the treatment plant, wastewater from nearby residential units, canteens and agricultural lands enters the drain. Physiochemical characterization of the wastewater samples revealed an average of 123.34±24.21 mg/L BOD, 214.86±32.14 mg/L COD, 35.95±6.91 mg/l NH_4-N, 6.58±1.23 mg/l PO_4-P and 12.34±3.80 mg/l NO_3-N. No pre-treatment unit was used in the present study as the drain mostly contained water runoff from nearby agricultural lands and did not have large particles. However, for wastewater containing coarse solid and other large particles, it is recommended to have pre-treatment using screening and settling units for removal of solids and debris, thereby avoiding clogging of the filter bed.

5.3.2. Site selection and soil quality assessment

In the present study, the mesocosm units were installed in green house which was 1-2 km away from the wastewater drain, resulting in additional requirement of manpower, water storage tanks and mode of transportation. It is advisable to install the wetland unit on site so as to reduce the cost incurred on the collection and transportation of the wastewater. Next, physical and chemical characterization of soil and biochar were done. Soil quality evaluation is important to estimate nutrient content, water retention capacity and soil compaction to minimize seepage into the groundwater. Soil capacity for water retention and infiltration will influence the plant growth and pollutant removal processes.

5.3.3. Selection of wetland components

Plants and substrate are two major components of the wetland system. Selection of the appropriate vegetation and substrate media is an important aspect of the wetland design as these components affect the physical, chemical and biological treatments processes taking place in the system thereby influencing the overall pollutant removal efficiency. While selecting the plant species, it is important to consider the location of the wetland and the type of wastewater. Apart from parameters like plant growth rate (fast or slow), root system (deep or shallow), seasonal growth pattern, and tolerance to flooding, sensitivity to anoxic conditions should be considered. Plants tolerance to wastewater stress and nutrient uptake capacity are also important considerations influencing the treatment performance. It is advisable to prefer native and adaptive plants varieties that the better suited to the local conditions. For the present study, native ornamental plants having good market value were selected in an attempt to create revenue generation opportunity apart from improving its aesthetic appearance (Fig. 5.12).

Canna indica, *Gerbera jamesonii*, *Lilium wallichianum*, and *Tagetes erecta* were grown in different substrate treatment combinations and were studied for their wastewater tolerance and flower production. The growth of *Canna indica* and *Lilium wallichianum* was more vigorous in all treatment combinations indicating plants were able to utilize nutrients for their growth. Both plants were found to thrive well in waterlogged conditions in all treatments. Flowering in *Canna indica* and *Lilium wallichianum* was observed in all treatment combinations indicating the availability of phosphorus to the plants. Sandoval-Herazo et al. (2018) reported that the growth of plants in the system is a good indicator of plant adaptation and their capacity to absorb nutrients from wastewater for their growth. To our knowledge, the use of *Lilium wallichianum* and *Tagetes erecta* plant species has not yet been reported in the constructed wetland system. Flowers of both plants are highly sought after in the local markets.

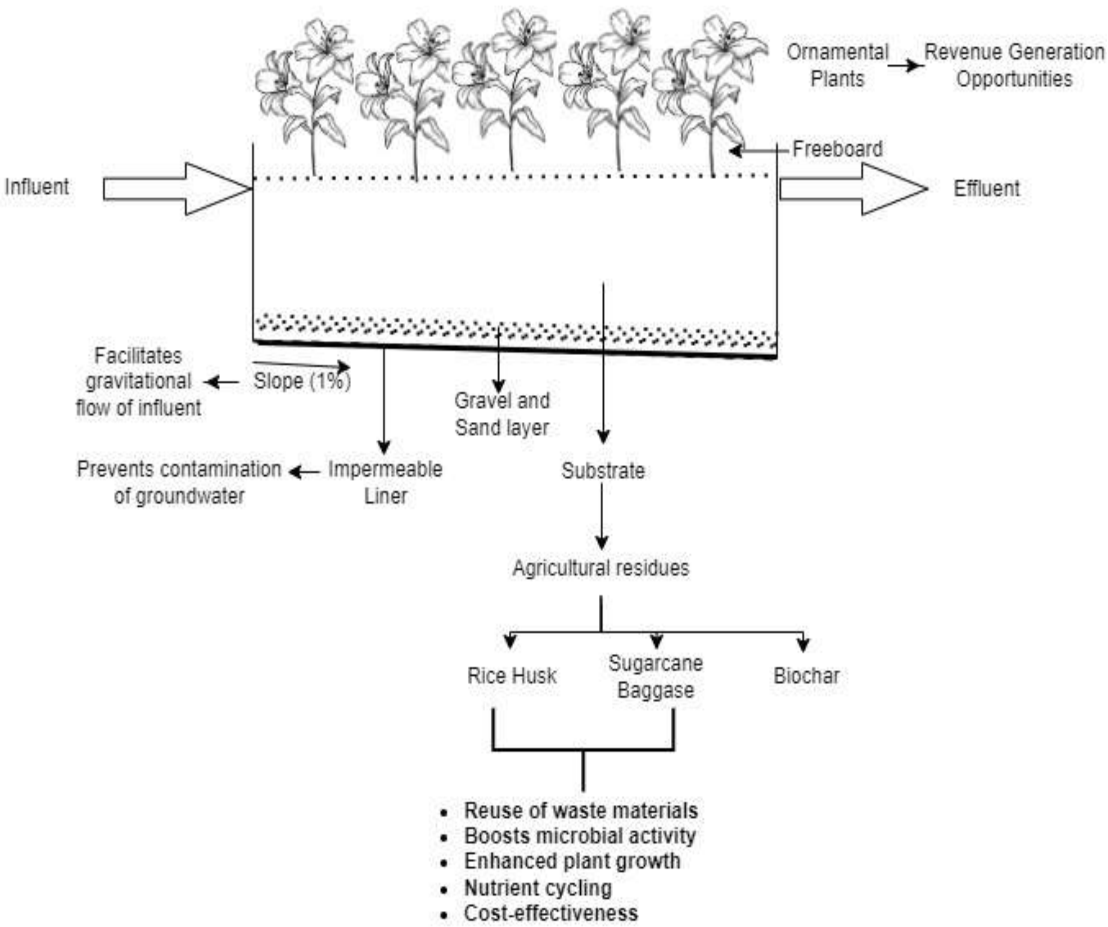


Fig. 5.12 Constructed wetland design specifications, substrate components and plants

The growth of plants in the wetland systems depends on their age, climatic conditions of the region as well as on the availability of nutrients in the wastewater. The effectiveness of the plants in the constructed wetlands is affected by the rhizospheric microbial communities and the interaction of these biotic components with the contaminants present in the wastewater (Ghosh and Gopal, 2010). Growth of the plant is also influenced by the pH of the medium which was observed to be on the higher side. In general, more biomass production and fast plant growth may be correlated with the higher removal of nutrients from wastewater (Calheiros et al., 2015).

Choice of substrates is usually influenced by its sorption capacity, permeability and nutrient removal efficiency. Soil is a conventional substrate used in constructed wetland systems along with sand and gravel. Increasingly different types of organic materials and industrial wastes are also finding increased application as wetland substrates. In the present study, agricultural wastes abundantly available in the region were explored for their potential as wetland substrates. Incorporating agricultural residues in these systems promotes reuse of waste material and makes the system more economical. Using readily available and cost-effective agricultural waste is an innovative and sustainable approach.

Wastewater is a rich source of nutrients like nitrogen and phosphorus which provide additional nutrients for plant growth. Nitrogen is a major component of proteins, nucleic acids, vitamins, and hormones and is primarily responsible for vegetative growth. Phosphorus is a vital nutrient involved in reproductive growth and flower production. Phosphorus also plays a pivotal role in photosynthesis, respiration, enhancing bud development, seed formation, and stimulating root growth. Various technologies to capture these essential nutrients from wastewater are increasingly being employed worldwide (Maroušek and Gavurová, 2022).

The main advantage of using lignocellulosic biomass as the substrate in the constructed wetland is its organic content and biodegradable nature. Rice husk and sugarcane bagasse have high silica content and silica is known to play an important role in the induction of resistance against the abiotic and biotic stresses in plants (Maroušek et al., 2022; Sharma et al., 2023). It's important to properly prepare and size the substrate material to optimize its effectiveness in the treatment process. Artificially prepared organic materials like biochar are also known to enhance overall soil fertility by improving soil structure, creating pores, boosting microbial communities, improving soil water management, and increasing the plant available water content in the soil. It also plays an important role in recycling and recovering nutrients like nitrogen and phosphorus (Maroušek and Trakal, 2022). Biochar is also shown to release carbon and thereby enhance denitrification (Yang et al., 2018).

Utilizing abundantly available, low-cost agricultural residues as components of the substrate for growing ornamental plants will make these systems more cost-effective as well as profitable. Conventional substrates (soil, sand, and gravel) used in constructed wetlands do not provide organic carbon. The incorporation of naturally occurring organic materials provides carbon sources for nitrogen removal. The incorporation of residues improves soil properties by increasing hydraulic conductivity, reducing bulk density, and acting as a reservoir for plant nutrients. Biological materials are also being explored for their ability to serve as nutrient sorbents. Nutrient sorption via biological materials like post-harvest residues and biochar can in fact turn them into fertilizer (Stávková and Maroušek, 2021).

In the study undertaken, rice straw, sugarcane bagasse, and biochar were used as substrate components which showed improved plant growth. Rice straw comprises biodegradable cellulose, hemicelluloses, recalcitrant lignin, and crude proteins. The addition of rice straw has been shown to increase total organic carbon and dissolved organic carbon in the soil and improve soil health. It is further shown to promote microbial growth by providing a large surface area for microbes to function and grow. Straw addition alters soil bacterial community and increases microbial biomass carbon. Adding rice straw to the constructed wetland is also shown to provide a large amount of organic carbon for microorganisms (Zhang et al., 2019). Saba et al. (2015) demonstrate the effectiveness of rice agricultural waste as substrate in constructed wetlands to treat dye-polluted water.

Sugarcane bagasse is a fibrous material comprising of cellulose, lignin, hemicelluloses, and chemical components like pentosans, ash, and α-cellulose. Bagasse has low density, good moisture content (45 to 55%), and a wide range of particle sizes (Saeed et al., 2018) making it a suitable substrate choice. The slow decomposition rate of these residues provides continuous labile organic carbon input in comparison to a liquid carbon source (Zhang et al., 2019; Feng et al., 2020). In the present study enhanced nutrient absorption was found in plant leaves which were consistent with the findings of Saeed and Sun (2013) who reported nitrogen removal by utilizing sugarcane bagasse and sylhet sand as a substrate for the treatment of textile wastewater. The study reported the efficiency of the bagasse material for oxygen transfer and facilitating denitrification in the system.

Further, biochar was also used as a substrate component, which is a stable solid, rich in carbon, and is widely used as an organic soil conditioned to enhance soil properties by improving soil's water-holding capacity and nutrient absorption. Biochar is also known to encourage the growth of nitrogen and phosphorus removing microorganisms. Biochar provides a large specific surface area for adsorption and improves the activity of wetland microorganisms (Wang H et al., 2020). Owing to its unique physicochemical characteristics, biochar is an excellent material for fertilization, soil refinement, carbon sequestration, and wastewater treatment. Nowadays, to lower production costs, majority of biochar is obtained by pyrolysis of biological wastes including postharvest residues (Maroušek and Trakal, 2022). In the present study, an accumulation of pollutants was observed in the substrate materials, which is consistent with the findings of Banitalebi et al. (2019) who reported that biochar is a porous carbonaceous material that can be used as a slow-release fertilizer and absorbent for pollutants.

5.3.4. Wetland configuration and working mechanism

Three most common criteria to characterize artificial wetland are flow path of wastewater, water hydrology and type of vegetation growing in it. Different types of systems can be operated in combination also i.e. like hybrid systems to use specific advantages of each and attain high treatment efficiency (Vymazal, 2018; Vymazal, 2005). In places where land availability is not a concern, horizontal wetland systems can be installed, and in places where land is limited vertical column based systems can be preferred. Often combination of different flow patters (horizontal and vertical) and multiple treatment cells are used to achieve better treatment performance. Different constructed wetlands based on its configuration, flow pattern and vegetation are shown in Fig. 5.13. The figure also shows various recent performances enhancement approaches like aeration and effluent recirculation that can be adopted to improve efficiency of constructed wetland systems.

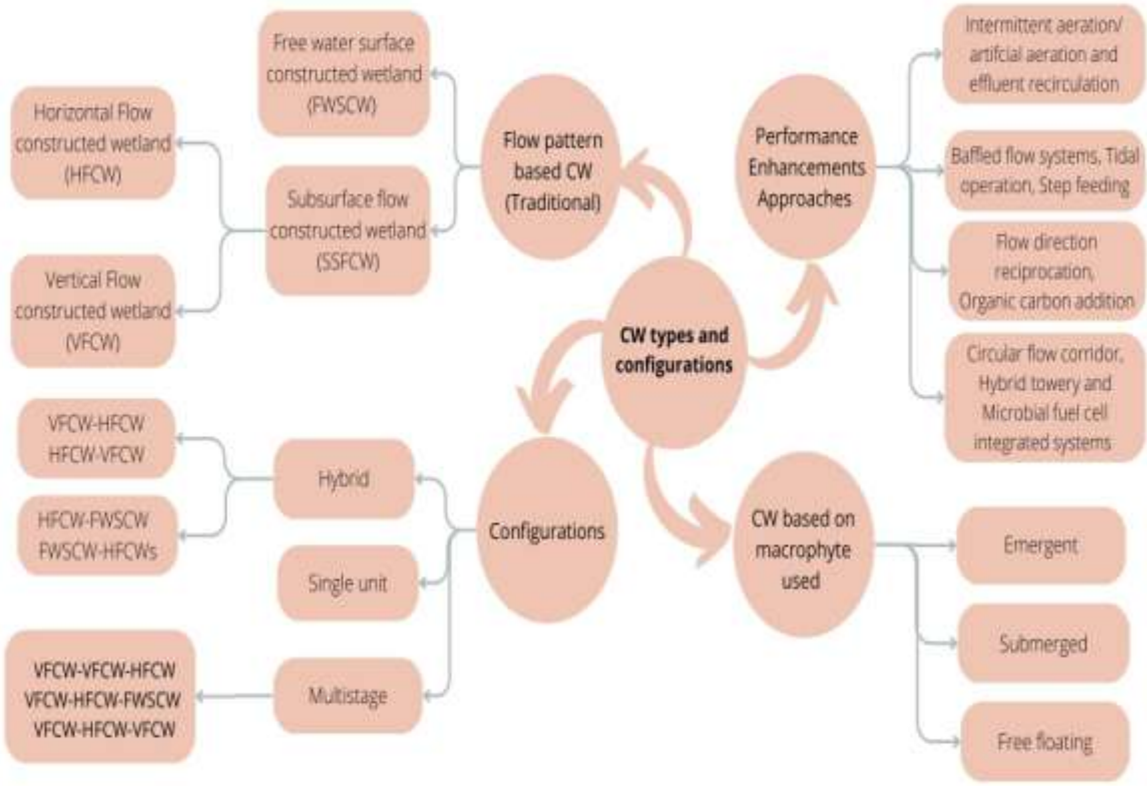


Fig. 5.13 Constructed wetland types and configurations based on flow pattern, cellular arrangement, macrophyte used and operational approach adopted

In the present study, vertical flow subsurface systems were used as it facilitated gravitational flow of influent. The influent was fed from the top of the unit and was collected through a faucet at the bottom of the mesocosm after a hydraulic retention time (HRT) of 24 hours. The wetland bed can be divided into three distinct zones i.e. inlet zone, treatment zone and outlet zone. Ideally, the influent should be distributed evenly throughout the entire length of the filter bed. Inlets structures are usually open end channel that can have single discharge point or multiple outlets as shown in Fig.5.14.

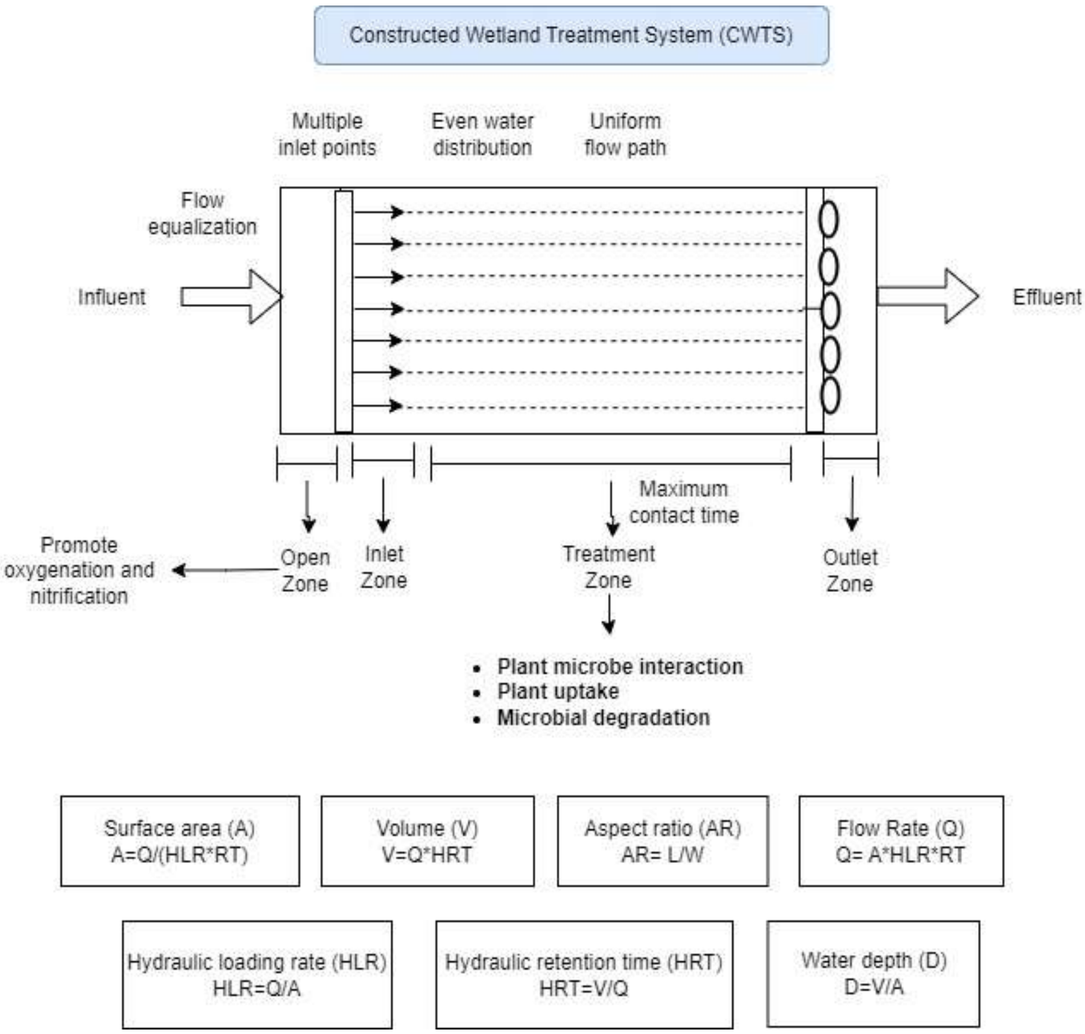


Fig.5.14 Influent flow pattern distribution, wetland zones and design aspects

Having multiple gated discharges promotes even flow distribution. In case the wastewater to be treated has high organic nitrogen or is not well oxygenated, an additional open zone before the vegetated bed can be created that will allow oxygenation and will also improve nitrification. In the inlet zone, wastewater enters the wetland bed and is evenly distributed across the filter bed. In the vegetated zone, roots provide active sites where microbial communities develop. Plant roots play a crucial role in oxygenation, nutrient uptake and supporting microbial populations. These microbial assemblages carry out transformation of nutrient and other organic compounds. Root structures take up nutrients like nitrogen and phosphorus from wastewater and in addition to this it also supplies oxygen to the areas adjacent to the roots and aids in aerobic degradation of contaminants.

Vegetation also provides carbon for denitrification during decomposition of biomass and it promotes removal of contaminants in anoxic conditions (Sandoval et al., 2019a). Plants can tolerate high nutrient concentration and may accumulate it. Plants and microbes both can uptake contaminants from wastewater. Engineered wetland systems are also referred to as root-zone treatment systems (RZTS). Root zone or rhizosphere is the active zone of CWs where all components of the system interact with each other and help in the removal of pollutants (Stottmeister et al., 2003). Processes involved in removal of different types of pollutants are shown in Fig. 5.15. Finally, after moving through the entire filter bed, the treated effluent exits the system from the outlet zone.

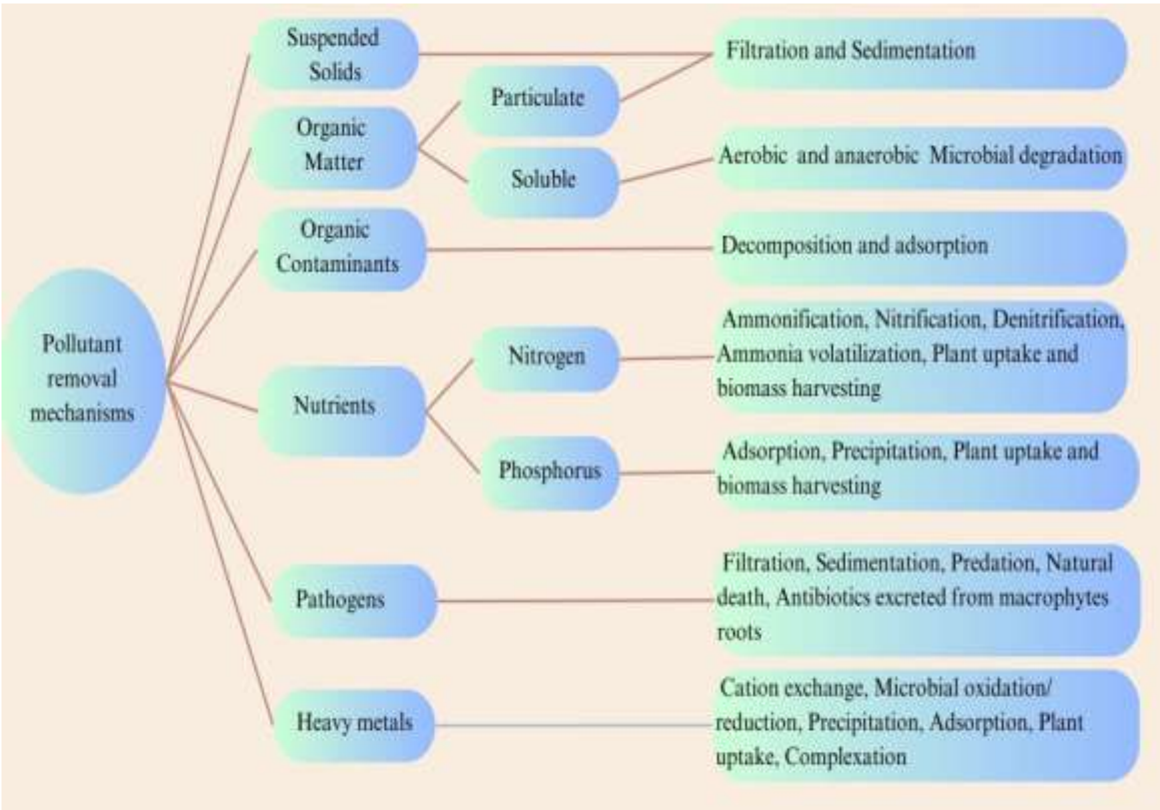


Fig. 5.15 Various processes involved in removal of different types of contaminants present in wastewater

5.3.5. Hydraulic factors

Hydraulic design is a crucial aspect that influences the overall functionality and sustainability of the constructed wetland treatment systems. Hydraulic loading rate (HLR) and hydraulic retention time (HRT) are important factors that influence the overall functionality of the system. Increasing the retention time and decreasing the loading rate have been shown to improve nutrient removal efficiency of the wetland. HRT ranging from 4 to 15 days have been reported to be most effective in such systems (Ghosh and Gopal, 2010). The average time for which water remains in the wetland i.e. retention time influences the pollutant removal effectiveness of the treatment system. Increasing contact time of the pollutants with the wetland components leads to improved pollutant removal. Various factors influencing the contact time of wastewater in CW thereby influencing the overall effectiveness are shown in Fig. 5.16.

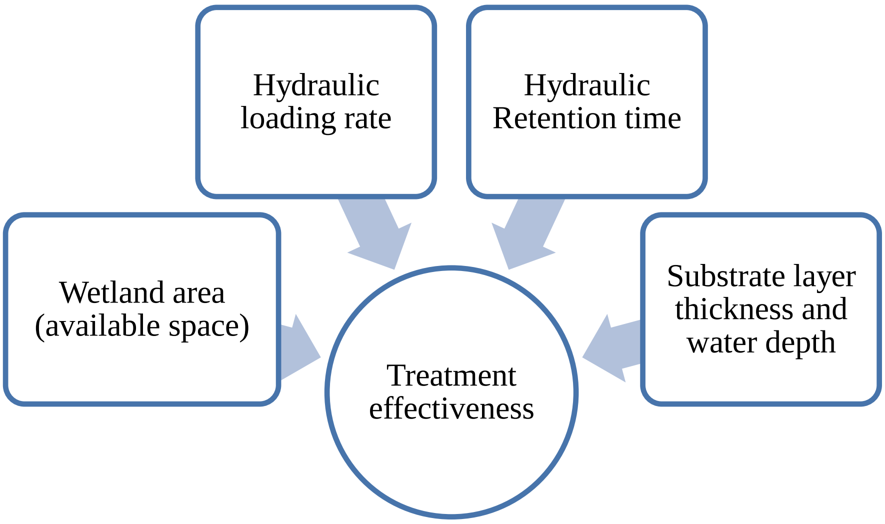


Fig.5.16 Factors affecting the contact time of wastewater in CW system

5.3.6. Treatment performance

After 24 hours retention time the removal efficiency of BOD was observed to be around 39%-58% and for COD it was 43%-65%. For nutrients removal efficiency was observed to be 40.64% for NH_4-N, 41.76% for NO_3-N, and 21.53% for PO_4-P. It is recommended to increase the retention time as it increases the contact time of the wastewater with the wetland components. Several studies carried out with higher retention times, have also reported improvement in the quality of the effluent as it will increase the contact time of the wastewater through the wetland components (Saeed et al., 2018). To improve treatment efficiency of the systems, retention time can be increased and effluent recirculation, aeration can also be used.

5.3.7. Cost benefit analysis and economic considerations

The most important factor on which adoption and implementation of these systems is based is the overall budget of designing and running a wetland system. It involves estimation of initial setup cost, regular operation and maintenance expenses (Fig. 5.17). Cost benefit analysis of any treatment method is important parameter to access its suitability. It usually involves cost incurred in acquisition of land, initial capital investment, regular operation, maintenance, chemical and energy consumption and the offered ecological benefits.

Initial capital investment	Land acquisition and preparation
	Pretreatment systems, wetland construction and installation
	Engineering and design expenses (hiring design engineers, wetland professionals and ecologists)
Maintenance and operation costs	Regular monitoring, water testing, vegetation harvesting, sediment removal, wastewater transport to the wetland unit
	Replacement and repair costs, upgrading monitoring equipments, repairing infrastructure
	Labor cost (construction workers, maintenance staff, specialized technicians)
Regulatory compliance	Environmental assessment and permits charges
	Water sample analysis and testing services

Fig.5.17 Components of the constructed wetland treatment systems overall budget

Constructed wetland is a cost efficient decentralized approach that offers flexibility in design with broad choice of substrate materials and plants that can be grown in the system. In the present study, locally available materials and native plants were utilized to design a constructed wetland, which considerably reduced the overall cost of the treatment unit. Agricultural waste was collected from farmland and sugar industry free of cost being a waste material. Biochar was procured from Greenfield

Eco Solutions, Jodhpur, Rajasthan, India at the nominal cost of INR 200 per Kg. Sand and gravel was obtained from construction site of civil engineering department, LPU. Greenhouse facility of the University was used to raise plants. The overall concept is realistic to scale up considering the low cost of the entire setup. To further reduce the wastewater collection and transportation expenses, the treatment unit can be installed close to the source.

One of the most limiting factor for the implementation and broader applications of the constructed wetland treatment systems is the land requirement especially in places that have high population density resulting in limited available land. In such cases innovation and improvements in the operation strategies and wetland design can be employed to achieve higher removal performance thereby increasing lifecycle cost of CW (Wu et al., 2015). Few recent studies have reported small scale treatment systems to be highly efficient in removal of pollutants. Additionally, incorporation of various effective design processes like artificial aeration, effluent recirculation, design and flow pattern, appropriate substrate and plants to enhance efficiency can reduce the excessive land requirement but will incur additional cost (Wu et al., 2015; Shukla et al., 2022).

While designing CWTS potential funding sources should also be considered. Grants and subsidies offered by government and other environment agencies can be used. Partnership with industries and institutions should also be explored. Although setting up CWTS requires budget but it comes with a significant return on investment i.e. ecological benefits offered against the cost incurred (Fig. 5.18). The benefits offered by the CWTS outweigh the costs thereby making the systems economically viable. In comparison to the traditional methods, CWTS offer numerous addition benefits like aesthetic value, carbon sequestration, recreational uses, wildlife habitat, flood control, groundwater recharge, aquaculture, fisheries etc (Kumar and Dutta, 2019)

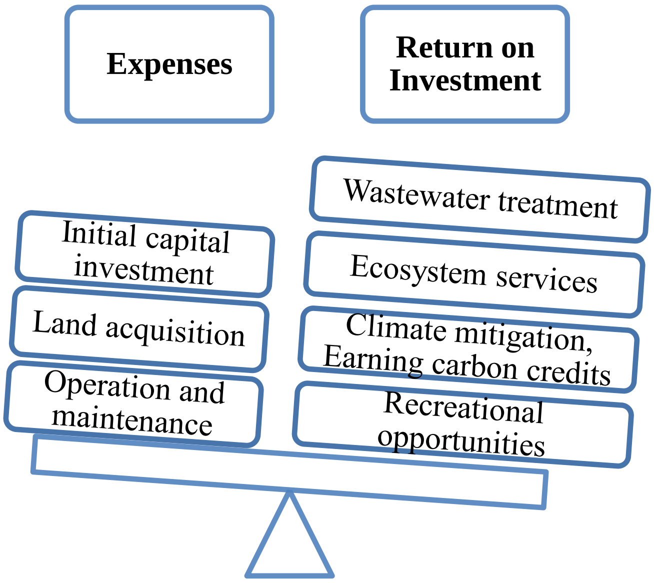


Fig.5.18 Comparison of constructed wetland treatment systems expenses and benefits

5.3.8. Sustainability of constructed wetlands

To ensure the effectiveness and sustainability of a constructed wetland for wastewater treatment, a number of criteria must be carefully taken into account while designing the treatment system. For instance, while designing constructed wetland treatment system, complex strategies should be avoided because as it may result in failure. Smooth operation and long-term performance will be ensured by simple design and uncomplicated processes. System should be designed to make use of the natural energies and processes instead of over-engineering. For instance, for wastewater flow, gravitational flow should be used instead of motors and control valves that require daily adjustments. The system should be designed in accordance to the natural topology of the location. The designed system should have minimal maintenance requirements. Appropriate time should be given to the designed system to mature i.e. become functional and attain its maximum removal efficiency. During this period, the plant primarily grows and the system develops a diversified microbial population and biofilm that carry out numerous transformations necessary for the removal of contaminants. Suitability of the designed system to work in harsh climatic and meteorological condition should also be assessed.

Another advantage that these systems offer is the flexibility in the mode of operation. These systems can be operated in both centralized and decentralized modes. Constructed wetland can be easily integrated with other existing treatment solutions as shown in Fig. 5.19. These can be combined with certain pre-treatment units like screens and septic tanks. Equalizations tanks can be used to regulate the variability of flow and pollutant load. Pre-treatment unit also helps to ensure long term operation by preventing clogging of the filter bed. Effluent from CWTS can also be circulated through tertiary treatment solutions like oxidation ponds and disinfection which will further improve the effluent quality. The treated effluent can be utilized for various agricultural, domestic, industrial and other purposes.

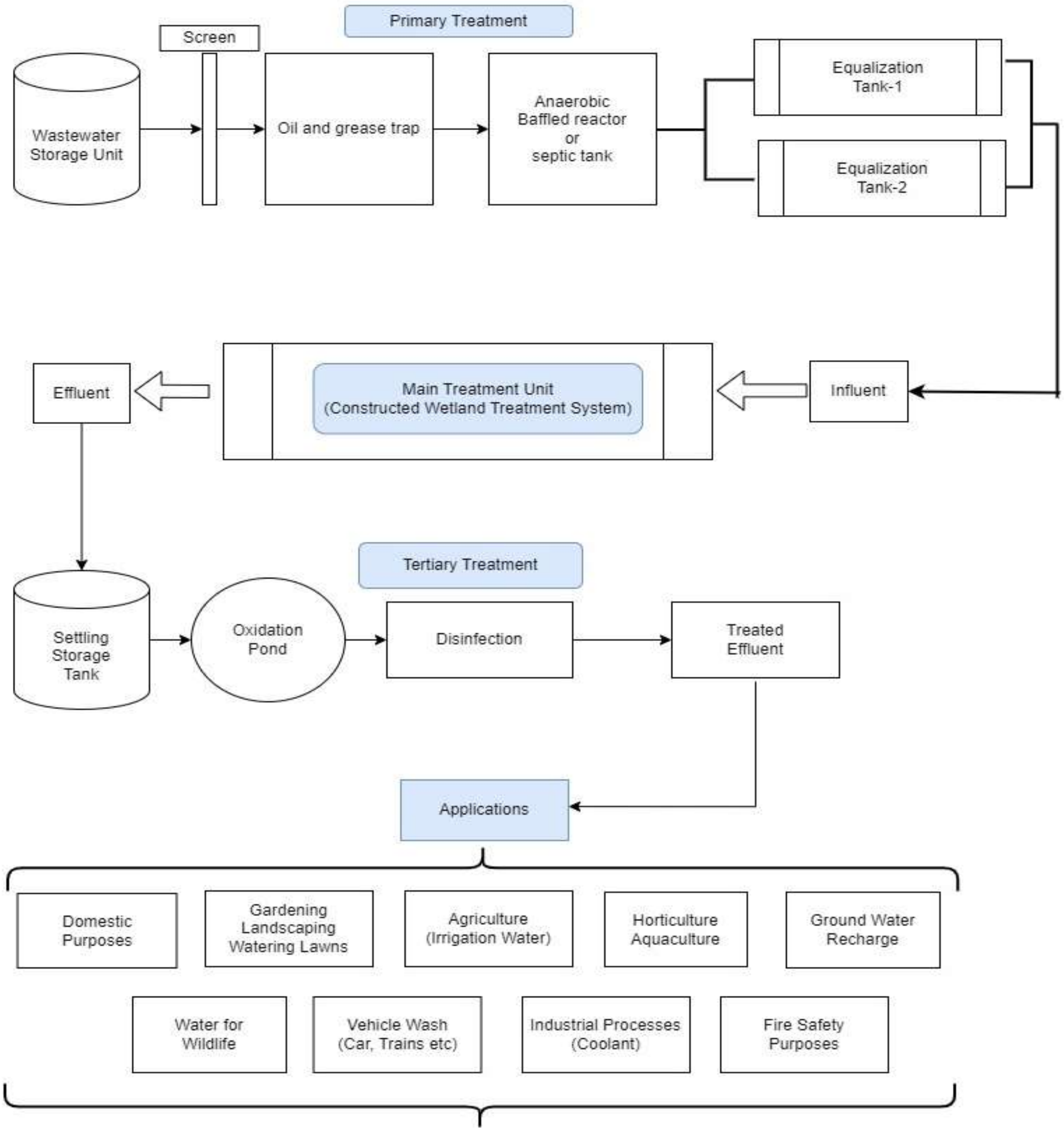


Fig. 5.19 Integrated constructed wetland treatment system and effluent reuse options

Chapter 6

Summary and Conclusions

6.1 Summary

The focus of the present study was to assess the performance of vertical sub-surface flow constructed wetland mesocosms having ornamental plant species grown in media amended with locally available agricultural and industrial by-product, for treatment of wastewater. Apart from the conventional media components (soil, sand, and gravel), two abundantly available lignocellulosic wastes in the region i.e. rice straw and sugarcane bagasse along with biochar were incorporated as wetland media components. Utilizing agricultural waste as substrate and growing commercially valuable ornamental plants, which will not only reduce the overall cost of these systems but will also make the system more profitable as well as easily integrable in the rural and urban landscapes. For selection of plants, native plant varieties were considered wherein the tropical climatic conditions of the region favored the growth of these plant species. In addition to improving the aesthetic appearance of the system, the commercial value of ornamental plants can be an additional plus and source of income, thereby promoting the use of constructed wetlands as wastewater treatment systems in developing nations, urban, and rural communities.

Four different ornamental plants of the family Cannaceae (*Canna indica*), Liliaceae (*Lilium wallichianum*), Asteraceae (*Tagetes erecta* and *Gerbera jamesonii)* were allowed to establish in the system for three months before starting the treatment phase. The propagation and establishment of the ornamental plant species were done successfully under wastewater applied conditions. The performance of ornamental plants and the media combinations were examined for their pollutant removal efficiency. Prepared substrate treatment combinations were found suitable for flowering in ornamental plants which is an indication of the availability of nutrients like phosphorus, which plays an important role in flowering, in the substrate matrix. Flowering was observed in all plants, except *Gerbera jamesonii* which was not able to adapt and produced no flowers. *Canna indica* and *Lilium wallichianum* have a greater potential of doing well in constructed wetlands and can be used in wetlands for revenue generation. However, studies over a longer period are recommended using the selected ornamental plants and substrates, on a pilot scale, to validate the treatment effect of the substrate. Various industries that produce biological waste like the sugar industry can set up a constructed wetland treatment plant on site, utilizing the generated waste as the substrate to grow plants and treat the wastewater.

The investigated mesocosms were found to be potentially effective in wastewater treatment with the removal efficiency of 39%-58% for BOD, 43%-65% for COD, 24%-53% for NH_4-N, 25%-55% NO_3-N and 12%-30% PO_4-P. The performance of the CW system investigated in this study can be further improved by experimenting with more than one retention time or recirculating the effluent until pollutant removal efficiency reaches greater than 70 to 80%. The study concluded that using agricultural residues and biochar as the substrate is a viable alternative as it provides additional organic carbon and nutrients in the system. The present study also demonstrates that ornamental plants are suitable alternatives to the commonly used plant species in CW systems such as *Phragmites* and *Typha*. In addition to flower production, above ground plant biomass can be harvested after regular intervals and can be used for alternative applications like energy generation, biogas, and biofuel production. Harvested biomass can also be converted into biochar which has the potential to be re-utilized as a pollutant absorbent or as a soil amendment.

In the present work, a comprehensive review of all aspects of designing a constructed wetland treatment system was done as design of the wetland system has a significant influence on its treatment performance and is a crucial factor in achieving the intended treatment objectives and associated ecological, social and economical advantages. Identified key considerations during each stage of the design process are presented in Fig. 6.1.

Constructed wetlands can be made more profitable by using low-cost, readily available agricultural waste as parts of the substrate for growing plants that are visually appealing. This will foster the adoption of these systems and strengthen their integration with existing wastewater treatment technologies. Utilizing rice straw, sugarcane bagasse, and biochar as substrate components in the present study demonstrated enhanced growth of the plants. Its organic content and biodegradable nature make lignocellulosic biomass an excellent choice for the substrate of the constructed wetlands. Rice husk and sugarcane bagasse have high silica content and silica is known to play an important role in the induction of resistance against the abiotic and biotic stresses in plants.

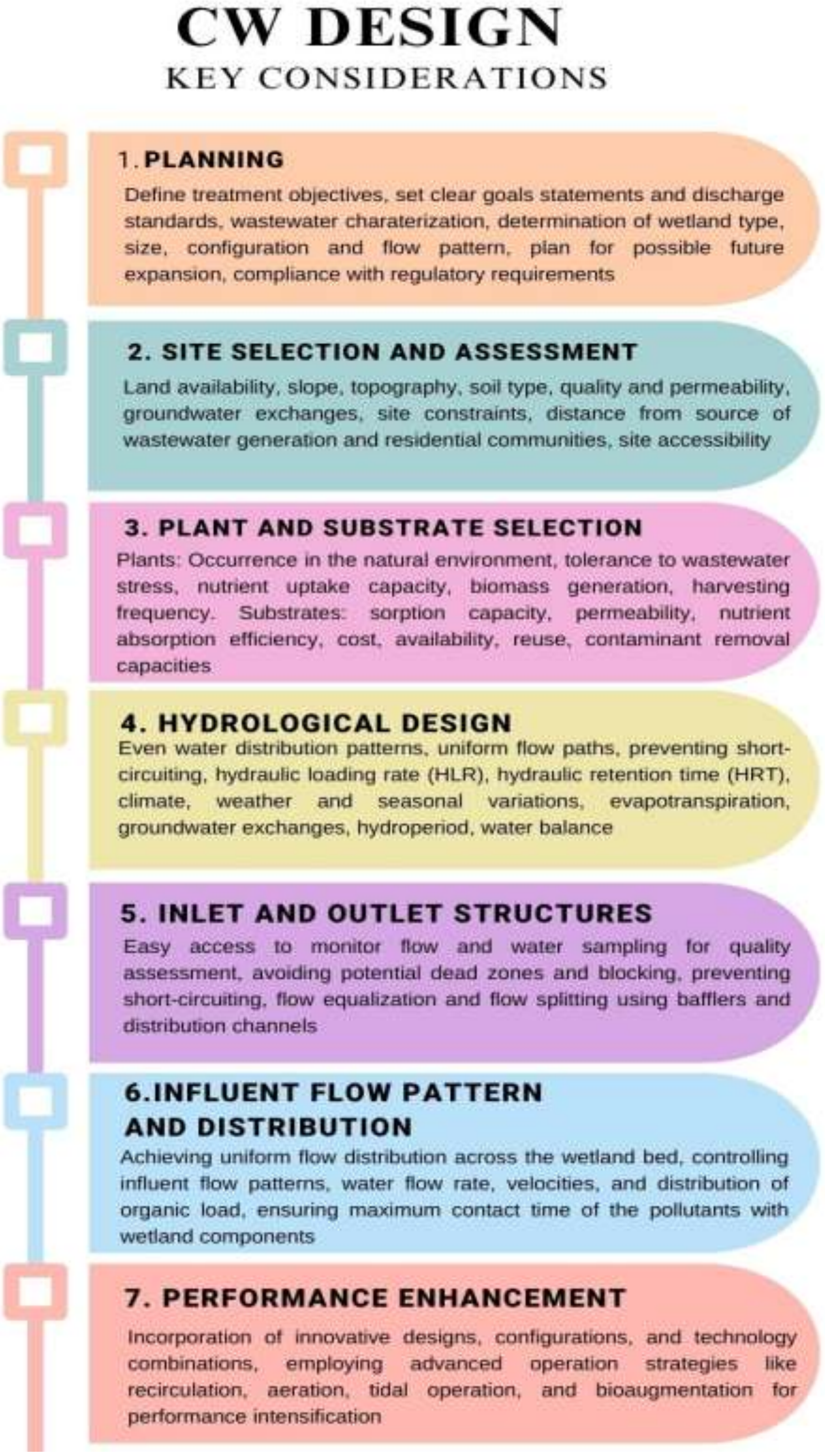


Fig.6.1 Key considerations during various stages of designing constructed wetland treatment systems

6.2 Environmental significance and future scope

Apart from wastewater treatment, thoughtfully designed wetland systems promote biodiversity by providing dynamic habitat to plants, animals, microbes and pollinators thereby supporting ecological balance of the environment. Well thought out design also provides other ecological benefits like acting as buffer against extreme weather events, reducing risk of flooding and preventing erosion by stabilizing soil. They can also play a role in carbon sequestration, climate change mitigation and adaptation. Well constructed systems can also be used for educational purposes and creating awareness about sustainable practices in water management and ecosystem restoration. These can also be used for recreational activities and tourism.

Results obtained in the present study demonstrated the potential of using lignocellulosic biomass in constructed wetland for plant growth; however, further studies are required to verify the treatment effect of the selected substrates on a pilot scale. Moreover, the influence of variations in the composition of the wastes and seasonal variations on the treatment effects of the units also needs to be further investigated. Presently few studies have highlighted the potential of using agricultural waste products in constructed wetland systems for their organic value and carbon source for nitrogen removal. In the future various other abundantly available agricultural wastes can also be evaluated for their potential to remove pollutants and promote plant growth.

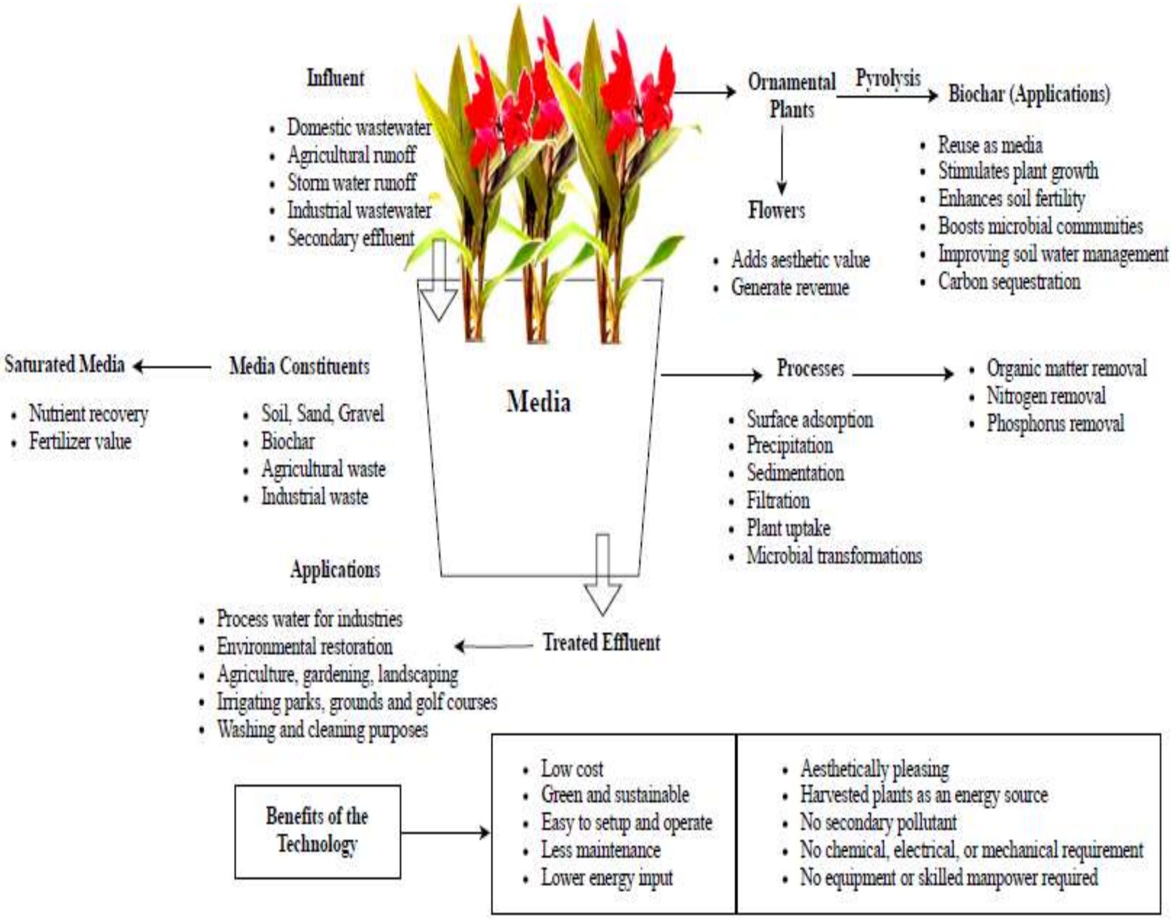


Fig.6.2 Various applications and benefits of constructed wetland mesocosm treatment systems

Incorporating abundantly available, low cost biowaste as part of wetland substrate not only helps with its disposal and reduces the wetland cost, but also provides an additional carbon source for microbes and acts as a reservoir for plant nutrients. It can further be reused as an organic fertilizer for soil amendment, carbon sequestration and improving soil health. Various applications and benefits of the constructed wetland mesocosm systems are shown in Fig. 6.2. Other abundantly available agricultural wastes can also be evaluated for their potential for pollutant removal and plant growth, but future studies are needed in testing and evaluating those materials. Future studies can also be targeted toward utilizing the saturated substrates for their fertilizer value. Techniques to recover valuable plant nutrients from the saturated substrates are another potential area to be investigated further.

This study provides an opportunity to explore ideas to market the saturated substrate for nutrient recovery and its use as a potential source of organic fertilizer at a much higher cost to promote sustainable vegetable and flower farming in urban areas of the world. Another idea could be to investigate the role of harvested plant biomass from the wetlands for biochar production which can be used to improve soil structure, enhance microbial communities, carbon sequestration, and to bring additional income to farmers. These systems also help to earn carbon credits apart from providing flowers to the local markets.

Future work can also focus on evaluating other design parameters and operational approaches for CW designs such as varying pollutant loading rates and hydraulic retention time for different types of media, using other plants compared to ornamental plants used in this study, and promoting CW as a climate mitigation and environmentally friendly practice for treating wastewater which is a major problem faced globally. Treating wastewater using nature based systems is an ideal way to convert wastewater into an additional source of water for human use, thereby increasing water security and contributing towards achieving sustainable development goals.

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List of Publications

1. **Patent**

   Republic of South Africa, Official Application no- 06984

   Title- Novel biowaste fertilizer for growth of ornamental plants for wetland system

   Granted- 28-Sep-2022

2. **Referred journal article**

   Sharma, M., Sharma, N.R. and Kanwar, R.S. Assessment of agriwaste derived substrates to grow ornamental plants for constructed wetland. Environ Sci Pollut Res 30, 84645–84662 (2023). https://doi.org/10.1007/s11356-023-28364-5 (Impact Factor- 5.8)

3. **Book chapter**

   Sharma M., Sharma N.R. (2020) Metagenomic Applications of Wastewater Treatment. In: Chopra R.S., Chopra C., Sharma N.R. (eds) Metagenomics: Techniques, Applications, Challenges and Opportunities. Springer, Singapore. https://doi.org/10.1007/978-981-15-6529-8_10

   Print ISBN978-981-15-6528-1

   Online ISBN978-981-15-6529-8

4. **Oral presentations**

a) E-conference on Post harvest Disease Management and Value Addition of Horticultural Crops by ICAR-IARI from AUGUST 18-20, 2021.

   Themes- Residue management and Value addition

   Abstract Title- Utilizing agricultural residues as substrate for growth of ornamental plants in Constructed Wetlands

b) International Conference on Recent Advances in Agriculture, Engineering and Biotechnology for Food Security by Mahima Research Foundation and Social Welfare on SEPTEMBER 25- 26, 2021.

   Themes- Environmental Biotechnology, Environmental Pollution, Greenhouse and Horticulture etc

Abstract Title- Potential of Ornamental Plants for Wastewater treatment in Constructed Wetland

c) International Conference on Sustainability, Life on Earth 2021 (ICS-LOE 2021) at Lovely Professional University, Punjab by Institute of Forest Productivity, Ranchi, Jharkhand on 17-18 December 2021

Registration number: ICSLOE20211084

Abstract Title **-** Selection of suitable substrate and plant for the constructed wetland treatment system

5. Poster presentations

a) 106th Indian Science Congress at Lovely Professional University, Punjab, 3rd January to 7th January 2019 (ISC106)

ISCA Membership Number: A1569

Poster Title- Potential of Biochar based retention systems for removal of Escherichia coli from wastewater

b) International Conference on Biosciences and Biotechnology at Lovely Professional University, Punjab, November 4th and 5th 2019 (ICBB2019)

Poster Title- Phytoremediation in removal of contaminants from wastewaters

c) International Conference on Clean Water, Good Health, Sustainable Cities and Communities at Lovely Professional University, Punjab, 18th and 19th October, 2023 (CWGHSCC)

Poster Title- Are nature-based solutions the key to attaining sustainable wastewater management and treatment?

d) International conference on microbial bioprospecting towards sustainable development goals at Lovely Professional University, Punjab, 24th-25th November, 2023 (ICMBSDG)

Poster Title- Constructed wetland plants derived biochar: A review of its untapped potential, applications and challenges

6. Workshops and short term courses

a) AICTE Training and Learning (ATAL) Workshops

   a. Advances in Waste Treatment Technologies

(August 23, 2021 to August 27, 2021)

b. Wastewater Treatment Recycle and Reuse

(September 6, 2021 to September 10, 2021)

c. Essentials of Academic Research

(November 8, 2021 to November 12, 2021)

d. Research Methodology and Data Analysis using SPSS and Tableau

(February 14, 2022 to February 18, 2022)

b) Workshops held in Lovely Professional University, Jalandhar

a. Authors Workshop on Scientific Writing

(March 25, 2023 to April 1, 2023)

b. Bibliometric Analysis, Systematic Literature Review and Meta-Analysis
(June 5, 2023 to July 12, 2023)

c) Short term course held in Dr. B.R. Ambedkar National Institute of Technology, Jalandhar

a. Advances in energy, environment and chemical engineering (AEECC- 2023)
(March 19, 2023 to March 23, 2023)