Efficiency of Constructed Wetland Microcosms (CWMs) for the Treatment of Domestic Wastewater using Aquatic Macrophytes with Special Reference to Nitrate and Phosphate Removal

THESIS

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

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Under the Supervision of

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Dedicated to

My Beloved Parents

and

Family members

CERTIFICATE

This is to certify that the thesis titled **"Efficiency of Constructed Wetland Microcosms (CWMs) for the Treatment of Domestic Wastewater using Aquatic Macrophytes with Special Reference to Nitrate and Phosphate Removal"** submitted by **Mr. Saroj Kumar** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other University.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) Regulations – 1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: 21.12.2021

Supervisor

Head of Department

(I/c)

DECLARATION

This is to certify that the material embodied in the present Ph.D. work titled **"Efficiency of Constructed Wetland Microcosms (CWMs) for the Treatment of Domestic Wastewater using Aquatic Macrophytes with Special Reference to Nitrate and Phosphate Removal"** is original research work done in fulfillment of requirements for the award of the degree of Doctor of Philosophy under the supervision of **Prof. Venkatesh Dutta**, Department of Environmental Science, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow-226025, U.P., India. It has not been submitted in part or full for any other diploma or degree in any other University or Institute. In this thesis, written content, data presented and plagiarism, if any, is the sole responsibility of the undersigned. If any allegations/query/question arises regarding the thesis, I, Mr. Saroj Kumar, will be solely responsible and answerable.

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“Sometimes writing is like driving through fog. You can’t really see where are you going. You have just enough of the road in front of you to know that you are probably still on the road, and if you drive slowly and keep your headlamp lowered you will still get where you were going...
And sometimes you come out of the fog into clarity, and you can see just what you are doing and where you are going which you could not see or know any of that five minute before”.

-Neil Gaimen

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Place: Lucknow

Date: 24/12/2021

Saroj Kumar

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PREFACE

This research work has been divided into four main objectives. The first objective of this work is to evaluate the removal efficiency of different constructed wetland microcosm (CWM) units for various water quality parameters including heavy metals (HMs) at dissimilar hydraulic retention times (HRTs) from domestic wastewater (DW) and factors affecting their performance. The selected physicochemical parameters such as biochemical oxygen demand (BOD), ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), total phosphorus (TP), soluble reactive phosphorus (SRP) and HMs such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As), nickel (Ni), zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) were analyzed for different CWM units at 3-, 7- and 14-days retention times. When the raw DW having these pollutants are discharged in a water body, it causes significant harm to aquatic life and deteriorates the quality of water in various ways as explained by several researchers throughout the globe. Measurement of BOD in DW specifies the amount of organic matter present in them. The presence of excess nutrients in wastewater causes eutrophication within the receiving water body by excess algal growth and develops red tides resulting in algal blooms (El-Sheekh et al., 2021; Wang et al., 2021). HMs within the environment are priority contaminants amongst the list of noxious elements due to their persistence and bioaccumulation ability (Ali et al., 2020). HMs, with high densities, are originating naturally, from construction materials, agriculture activities, industrial processing and transportation etc. However, in DW these might come from household activities, small industrial operations and urban runoff (Deng et al., 2021). The majority of the HMs pose toxicity even at lesser fractions and their concentrations in tissues over a time period could be harmful to ecosystems and human health (Titilawo et al., 2018; Agoro et al., 2020). The excess concentration of these heavy metals in the environment may cause toxicity to living beings that disrupt metabolic functioning. The concentration of these HMs within DW varies among cities or even for the same city depending upon residential and commercial locations and runoff characteristics.

As second objective, I have studied the interspecific competition as competitive value (CV) and relative growth rate (RGR) between selected macrophytic groups with pollutants removal efficiency. Further, several growth-related parameters of macrophytes such as the number of macrophytes, the total number of macrophytes, total dry biomass production, above-ground biomass (AGB), below-ground biomass (BGB) and root length per unit were studied to distinguish the dominant nature of the macrophytes. *Typha latifolia* was recognized as a superior competitor in competition with both *Phragmites karka* and *Pistia stratiotes,* due to their aggressive competitive nature that constrains the growth and development of adjoining macrophytes in a mixed culture. *Phragmites karka* was identified as the superior competitor when planted with *Pistia stratiotes* and inferior with *Typha latifolia.* However, *Pistia stratiotes is* observed as a weaker competitor against both the macrophytes. The negative CV of *Pistia stratiotes* with *Phragmites karka* and *Typha latifolia* explained that the overall biomass of *Pistia stratiotes* in monoculture was higher as compared to the mixed culture of these macrophytes. Similarly, *Phragmites karka* displayed negative CV with *Typha latifolia.* It is also due to the higher biomass of *Phragmites karka* in monoculture as compared to mixed culture. The relative growth rate of *Typha latifolia* was approximately two times greater than that of *Phragmites karka* among all experimental CWM units.

As the third objective of this research, I have assessed the vertical and temporal variations among the activity of different enzymes and their relationship with contaminant's removal efficacy within all CWM units. The removal efficacy of several selected water quality parameters was also studied. The outcome of this work exhibited that the enzyme's activity and contaminants elimination efficacy differ greatly depending upon retention time, substrate depth, type of contaminants and CWM units. The vertical variation exhibited that the top layer (0-10 cm) of CWM units have significantly the maximum activity of all selected enzymes and varied significantly from the lower layer. However, temporal variation exhibited significant variations over time with higher activity in May, June, and October for most of the enzymes. CWM unit planted with *Phragmites karka* and *Pistia stratiotes* showed higher enzymes activity in the top as well in the deeper layer. The correlation results showed that phosphatase activity was significantly linked with the exclusion of TP and SRP in maximum CWM units. The activity of urease was significantly positively correlated with NH_4^+-N removal in CWM units Pi+Ph+T and Pi+Ph. The activity of urease was also observed to have both negative and positive associations with the exclusion efficacy of NO_2^--N and NO_3^--N in various CWM units. However, the activity of the dehydrogenase (DHA) enzyme expressed a negative association with the removal efficiency of BOD excluding in CWM units Pi+Ph and Pi+Ph+T. Elimination of BOD and microbial biomass carbon (MBC) also expressed negative association with each other in the majority of the CWM units. However, a moderate positive and negative relationship was observed between BOD removal and fluorescein diacetate (FDA).

The fourth objective of this research work was to study the potential of CWs as polishing of DW after treatment with Upflow Anaerobic Sludge Blanket Technology (UASB) and Fluidized Aerobic Bed reactor (FAB) technology and their integration with these treatment systems for achieving discharge standards. The UASB reactors have been extensively used for the treatment of DW in several countries including India. Around 80% of the total worldwide installed UASB reactors for municipal wastewater treatment are present only in India. In the current work, it is evaluated that the removal efficacy of UASB reactors for several wastewater contaminants is enhanced significantly by integrating them with CWs as a post-treatment facility. It is evaluated that the concentration of BOD in the effluent of different CWM units after three days retention time successfully meets the discharge criteria of inland surface water. The maximum removal performance of several contaminants was expressed by the CWM unit Pi+Ph. However, the maximum removal of BOD via UASB reactor was recorded in Noida (79 ± 0.89%) having the treatment capacity of 34 MLD followed by the Kanpur (72 ± 1.41%). From these results, it is evident that the UASB or FAB-based reactors alone have not achieved water quality up to satisfactory discharge standards. However, the removal capability of the UASB reactor in integration with CWs in terms of BOD reached the highest (up to 98%) as compared to other available treatment technologies from DW. The removal of chemical oxygen demand (COD) (90%), total suspended solid (TSS) (92%), TN (89%), NH_4^+-N (70%) and TP (88%) were also higher.

LIST OF ABBREVIATIONS & SYMBOLS

°C	:	Degree Celsius
A	:	Final Concentration of HMs
A_0	:	Initial Concentration of HMs
AGB	:	Above-ground Biomass
Al	:	Aluminum
ANAMOX	:	Anaerobic Ammonium Oxidation
ANOVA	:	Analysis of Variance
AOB	:	Ammonium Oxidizing Bacteria
APHA	:	American Public Health Association
As	:	Arsenic
ATCF	:	Aerial Tissue Concentration Factor
BCF	:	Bioconcentration Factor
BGB	:	Below-ground Biomass
BOD	:	Biochemical Oxygen Demand
BOFS	:	Basic Oxygen Furnace Slag
Ca	:	Calcium
C_A	:	Concentration in Aerial parts of Macrophytes
CANAN	:	Complete Autotrophic Nitrite Removal Over Nitrate
Cd	:	Cadmium
Ce	:	Effluent Concentration
CEPT	:	Chemically Enhanced Primary Treatment
CETPs	:	Common Effluent Treatment Plants
CH_4	:	Methane
Ci	:	Influent Concentration
cm	:	Centimeter
CO_2	:	Carbon dioxide
COD	:	Chemical Oxygen Demand
CPCB	:	Central Pollution Control Board
Cr	:	Chromium
C_R	:	Concentration in Roots of Macrophytes
Cs	:	Concentration in Substrate
Cu	:	Copper

CV	:	Competitive Values
CWM	:	Constructed Wetland Microcosm
CWs	:	Constructed Wetlands
d	:	Day
DHA	:	Dehydrogenase
DHS	:	Down-flow hanging sponge
DO	:	Dissolved Oxygen
DW	:	Domestic Wastewater
DWP	:	Duckweed Pond
EAF	:	Electric Arc Furnace
EC	:	Electrical Conductance
EPS	:	Extracellular Polymeric Substances
FAB	:	Fluidized Aerobic Bed Reactor
FAS	:	Ferrous Ammonium Sulphate
FC	:	Faecal Coliform
FDA	:	Fluorescein Diacetate
Fe	:	Iron
Fe-GAC	:	Iron Oxide Granular Activated Carbon
H_2O	:	Water
ha	:	Hectare
HFCWs	:	Horizontal Flow Constructed Wetlands
HLRs	:	Hydraulic Loading Rates
HMs	:	Heavy Metals
HRTs	:	Hydraulic Retention Times
k	:	Rate Constant
Kg	:	Kilogram
LH-PCR	:	Length Heterogeneity Polymerase Chain Reaction
LIC	:	Low-Income Countries
LMIC	:	Lower-Middle-Income Countries
m	:	Meter
MBC	:	Microbial Biomass Carbon
Mg	:	Magnesium
mg/L	:	Milligram per liter
MION	:	Magnetic Iron Oxide Nanoparticle

MLD	:	Million Liters per Day
Mn	:	Manganese
MOEFCC	:	Ministry of Environment Forest and Climate Change
N_2	:	Nitrogen
NH_3	:	Ammonia
NH_4^+-N	:	Ammonium
Ni	:	Nickel
NO_2^--N	:	Nitrite- Nitrogen
NO_3^--N	:	Nitrate- Nitrogen
NOB	:	Nitrate Oxidizing Bacteria
NWP	:	National Water Policy
O&M	:	Operation & Maintenance
O_2	:	Oxygen
OH^-	:	Hydroxyl Ions
Pb	:	Lead
PCR-DGGE	:	Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis
Ph	:	*Phragmites karka*
pH	:	Power of Hydrogen
Pi	:	*Pistia stratiotes*
p-NPP	:	para -Nitrophenol Phosphate
PO_4^{3-}	:	Orthophosphates
PP	:	Polishing Ponds
RCF	:	Root Concentration Factor
RE	:	Removal Efficiency/ Efficacy
RGR	:	Relative Growth Rate
RH	:	Relative Humidity
RL	:	Root Length
ROL	:	Radial Oxygen Loss
rpm	:	Rotation per minute
RVFW	:	Recirculating Vertical Flow Wetland
SBR	:	Sequential Batch Reactor
SD	:	Standard Deviation
SDGs	:	Sustainable Development Goals
SFCWs	:	Surface Flow Constructed Wetlands

SPSS	:	Social Science Package for Statistical Analysis
SRP	:	Soluble Reactive Phosphorus
SSFCWs	:	Subsurface Flow Constructed Wetlands
STP	:	Sewage Treatment Plants
t	:	Time
T	:	*Typha latifolia*
TDS	:	Total Dissolved Solids
TF	:	Translocation Factor
TN	:	Total Nitrogen
TOP	:	Total Organic Phosphorus
TP	:	Total Phosphorus
TPF	:	Triphenyl Formazan
TSS	:	Total Suspended Solids
TTC	:	Triphenyl Tetrazolium Chloride
UASB	:	Upflow Anaerobic Sludge Blanket Technology
UNPD	:	United Nation Population Division
USEPA	:	United States Environmental Protection Agency
UV	:	Ultraviolet
VFCWs	:	Vertical Flow Constructed Wetlands
YAP	:	Yamuna Action Plan
yr	:	Year
ZON	:	Zirconium Oxide Nanoparticle
Zn	:	Zinc
α	:	Alpha
β	:	Beta
γ	:	Gamma
%	:	Percentage

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Chapter 1
Introduction

1.1 Introduction

Around 55.3% of the world's population is residing in the cities currently and is expected to reach upto 60% by 2030 (United Nations, 2018; Vij et al., 2021). Due to rapid expansion in urban settlements, the household water use has multiplied several folds and the gap between water demand and supply has amplified in metropolitan municipalities (Florke et al., 2013). Moreover, climate change and over-abstraction of groundwater is exerting further pressure and will exacerbate the upcoming water concern throughout the world (Asoka et al., 2017; Florke et al., 2018). The emerging situation of freshwater crisis can be seen clearly in metropolitan cities and is also evolving in the medium-sized and smaller cities (Aartsen et al., 2018). It is estimated that countries in the lower middle-income range (LMIC) are able to treat only 28% of their total wastewater produced and the lower income countries (LIC) have only 8% treatment capability (Sato et al., 2013). Low treatment capability of LMIC and LIC are primarily due to the high-cost investment in the treatment of wastewater and priority towards management of their solid waste (Kumar and Tortajada, 2020). The urban population of India has increased from 17.9 % (1960) to 34% (2018) as described by United Nation Population Division. It is expected that by 2030, the 6 Indian megacities might have 10 million or more residents, 13 cities with more than 4 million residents and 68 cities with more than one million residents (Vij et al., 2021). LMIC like India has only about 37% of total wastewater treatment capacity (Vij et al., 2021). Some top Indian megacities such as Chennai, Bangalore, Delhi and Mumbai are now facing serious water shortage, because of groundwater depletion and contamination through untreated sewage, storm water runoff and solid wastes (Jambeck et al., 2015). The water-deficient municipalities in these cities will find it very tough to gain ecological eminence and attain economic growth to achieve to the sustainable development goals (SDGs). It is well established that wastewater treatment and subsequent reuse is critical to reaching SDG 6 and 11 for clean water and sanitation and sustainable cities and communities respectively. Metropolitan cities in India such as Chennai and Mumbai have faced life-threatening drought conditions. Several policies have been framed in the last three decades to advance wastewater treatment and their reuse. However, various research reports have showed that there is inadequate capacity to handle wastewater and their possible reuse (Pandit and Biswas, 2019; Kumar and Tortajada, 2020). The key water policies associated with water and wastewater management are framed in India to safeguard precious water resource and to provide clean water to everyone. Some of them are Water Pollution Act, 1974, National Water Policy, 1987, National River Conservation Plan, 1995, Delhi Water Tariff Policy, 2009, National Water Mission Vol. 1, 2011, Draft Water Policy of Delhi, 2016, National Water Framework Bill, 2016, Draft Policy on National Urban Faecal Sludge and Septage Management, 2017 and National Status of Wastewater generation and Treatment, 2019 etc.

The National Mission for Clean Ganga (2011) was launched with goals to establish STPs in the entire Ganga basin in India. In this mission, 97 sewerage schemes along the Ganga River covering cities such as Kanpur, Allahabad, Patna and Kolkata have been employed. The new National Water Policy was launched in 2012 to address challenges originating from water shortage and climate change. One of the main objectives of this policy was to reuse and recycle urban wastewater originating from industrial and residential areas after treatment. Recently, '*Namami Gange'* project is trying to achieve the twin objectives of effective abatement of pollution as well as rejuvenation and conservation of River Ganga. The eight major goals of this project are to develop sewerage treatment infrastructure, river-surface cleaning, afforestation, industrial effluent monitoring, biodiversity conservation, development of river front and Ganga gram and public awareness. The status of water availability on the earth has been provided in fig. 1.

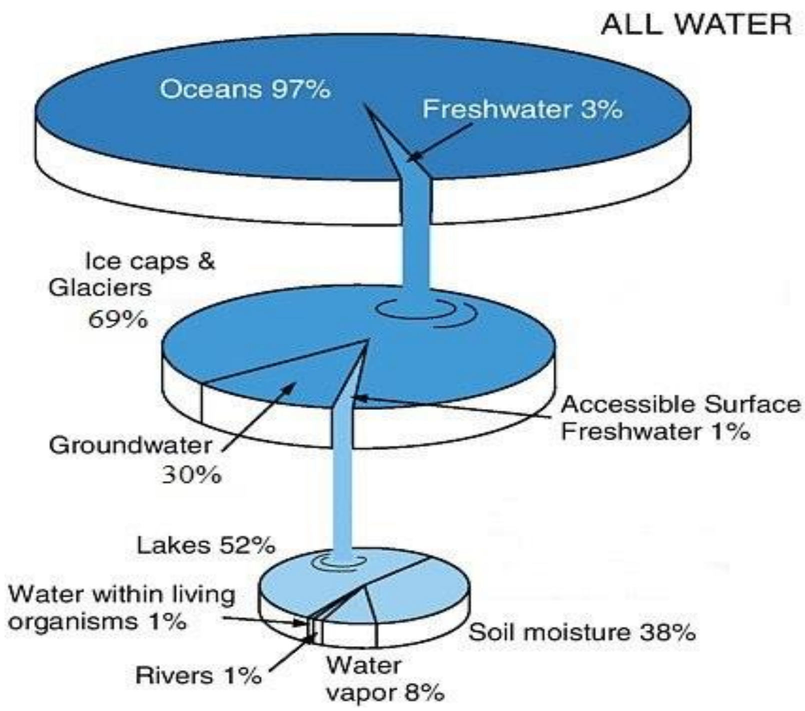


Fig. 1.1 Water profile on the earth (Modified from Uppu, 2020)

1.2 Wastewater generation and treatment – present status in India

Wastewater generated from domestic sources are discharged after partial treatment or directly routed to the nearby waterbodies. In India, about 61,948 MLD wastewater is generated from cities and towns whereas the existing sewage treatment ability is only 23,277 MLD (Roy, 2020). The status of wastewater generation in different states of India has been provided in fig. 1.2. This large gap between generation and treatment is because of low treatment capability, insufficient sewage networks, lack of technology and clear policy directions to improve wastewater reuse (MOEFCC, 2018). However, the present treatment capability is also not employed efficiently due to the operational and maintenance failures. Almost, 39% treatment plants are not in compliance with the discharge criteria as per the CPCB's reports. It is reported that 35 metropolitan cities (population more than 10 Lacs) generated approximately 15,644 MLD of sewage while the existing treatment ability is only 8040 MLD. Amongst the metropolitan cities, Delhi poses highest treatment capability of 2330 MLD (about 30% of total metropolitan cities). After that, Mumbai has 2130 MLD total treatment capacity which is 26%. The treatment capacity of various cities such as Chennai, Hyderabad, Ludhiana, Ahmedabad and Vadodara are meeting the volume of generation. Wastewater generation and treatment ability of all Indian states and union territories as provided by National Inventory of Sewage Treatment Plants has been provided in table 1.1.

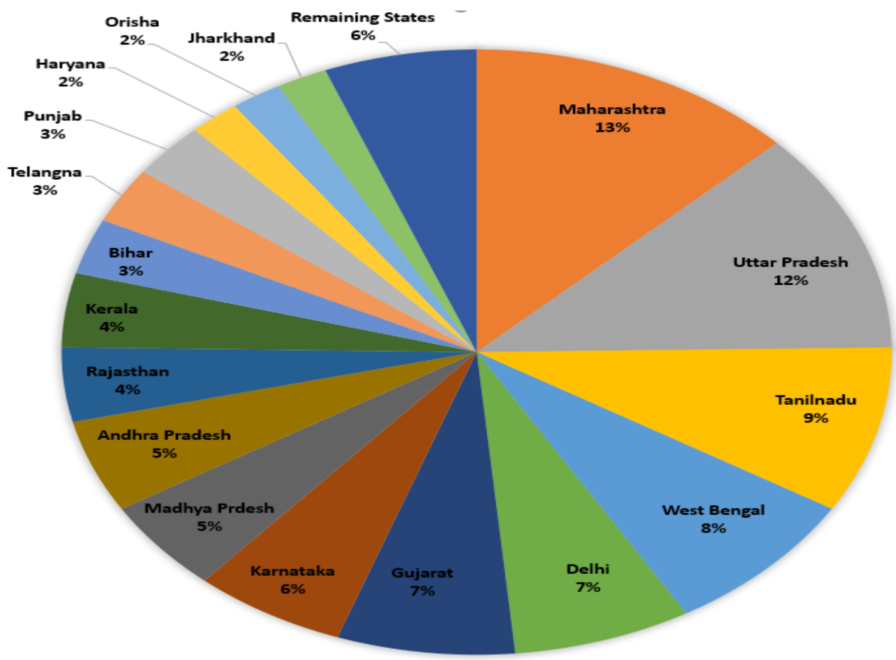


Fig. 1.2 Percent wastewater generation in different states of India (CPCB, 2016)

Table 1.1 Wastewater generation and treatment ability of all Indian states and union territories as provided by National Inventory of Sewage Treatment Plants (CPCB, 2021)

Sl. No.	Union Territory/ State	Sewage generated, (MLD)	Existing treatment capacity (MLD)	Proposed treatment capacity (MLD)	Operational treatment capacity (MLD)	Total no. of STPs	Total no. of CETPs
1	Andhra Pradesh	2882	833	20	443	66	11
	Assam	809	0.21	0	0	5	-
2	Arunachal Pradesh	62	0	0	0	-	-
3	Andaman and Nicobar Islands	23	0	0	0	8	-
4	Bihar	2276	10	621	0	1	-
5	Chhattisgarh	1203	73	0	73	3	-
6	Chandigarh	188	293	0	271	7	-
7	Daman & Diu	29	-			3	-
8	Dadra & Nagar Haveli	67	24	0	24		-
9	Gujarat	5013	3378	0	3358	70	30
10	Goa	176	66	38	44	11	-
11	Himachal Pradesh	116	136	19	99	78	1
12	Haryana	1816	1880	0	1880	153	14
13	Jharkhand	1510	22	617	22	2	1
14	Jammu & Kashmir	665	218	4	93	24	1
15	Kerala	4256	120	0	114	7	5
16	Karnataka	4458	2712	0	1922	140	9
17	Lakshadweep	13	0	0	0	0	-
18	Maharashtra	9107	6890	2929	6366	154	27
19	Madhya Pradesh	3646	1839	85	684	126	1
20	Meghalaya	112	0	0	0	-	-
21	Manipur	168	0	0	0	-	-
22	Mizoram	103	10	0	0	1	-
23	Nagaland	135	0	0	0	-	-

24	NCT of Delhi	3330	2896	0	2715	38	13
25	Odisha	1282	378	0	55	14	-
26	Punjab	1889	1781	0	1601	119	4
27	Puducherry	161	56	3	56	3	-
28	Rajasthan	3185	1086	109	783	64	14
29	Sikkim	52	20	10	18	6	-
30	Telangana	2660	901	0	842	37	-
31	Tamil Nadu	6421	1492	0	1492	63	49
32	Tripura	237	8	0	8	1	-
33	Uttarakhand	627	448	67	345	71	4
34	Uttar Pradesh	8263	3374	0	3224	107	8
35	West Bengal	5457	897	305	337	50	1

Wastewater released from decentralized locations contains higher concentration of organics, nutrients and heavy metals that pose great challenges on the removal mechanisms (Wojciechowska et al., 2017). Various traditional treatment methods such as ASP, UASB, membrane bioreactors, FAB and membrane separation etc. have been successfully applied to manage DW. Because of high-cost investment, the usage of such technologies is not adequate on field scale (Zhang et al., 2020). Constructed Wetlands Microcosms (CWMs) are applied for the treatment of several categories of wastewaters such as municipal, industrial, stormwater runoff and agricultural runoff etc. (Li et al., 2017; Kumar and Dutta 2019b; Kumar et al., 2020c). The use of this technology is considered eco-friendly and requires less operational and maintenance cost. They are also very efficient in the elimination of a variety of wastewater contaminants. CWMs are engineered ecosystems that has grown as an advanced system to tackle wastewater particularly generated from household activities. CWMs are well known for their eco friendliness, reliable efficiency, low energy inputs and easier operation (Kumar et al., 2020a). They utilize natural ongoing processes of macrophytes, substrate materials and available microbial populations to treat wastewaters. The use of this technology has grown several folds recently (Gupta et al., 2020). There are several co-benefits of CWMs along with wastewater management as they provide significant environmental amenities such as reuse, valuable wildlife habitat, groundwater recharge, flood control, carbon sequestration, silt capture, fisheries, recreational uses and aesthetic values. Presently, the use of CWMs has been extended globally due to the upgradation in their design and working parameters. CWMs offer a more attractive wastewater treatment technology as compared to traditional methods (De Rozari et al., 2018). Several ecological and working factors are vital for the proper and effective operation of CWMs, in which, the ecological factors like DO, pH and temperature more crucial. Working factors such as macrophyte choice, suitable design, HRT, site, C/N ratio, feeding mode, artificial aeration, wastewater recirculation, bioaugmentation of bacterial communities, suitable macrophyte harvesting and hydraulic loading rates (HLR) are also very critical. The vegetation present within wetland systems lessen the water velocities and make available adherence sites for microbial populations. However, after the decay of macrophytes or parts, litter offers extra carbon and nutrients essential for microbial growth for further action.

1.3 CWMs

An operational unit of CWMs holds numerous kinds of macrophytes, substrate materials and microbial assemblages (Fig. 1.3). In general, the wastewater passes within the treatment column, moves through the substrate materials and the effluent discharged out from an outlet. Basically, a CWM unit comprises five major components- these are a treatment basin, substrates, vegetation mainly macrophytes and an inlet and outlet structure (Sudarsan et al., 2015; Kumar et al., 2019b).

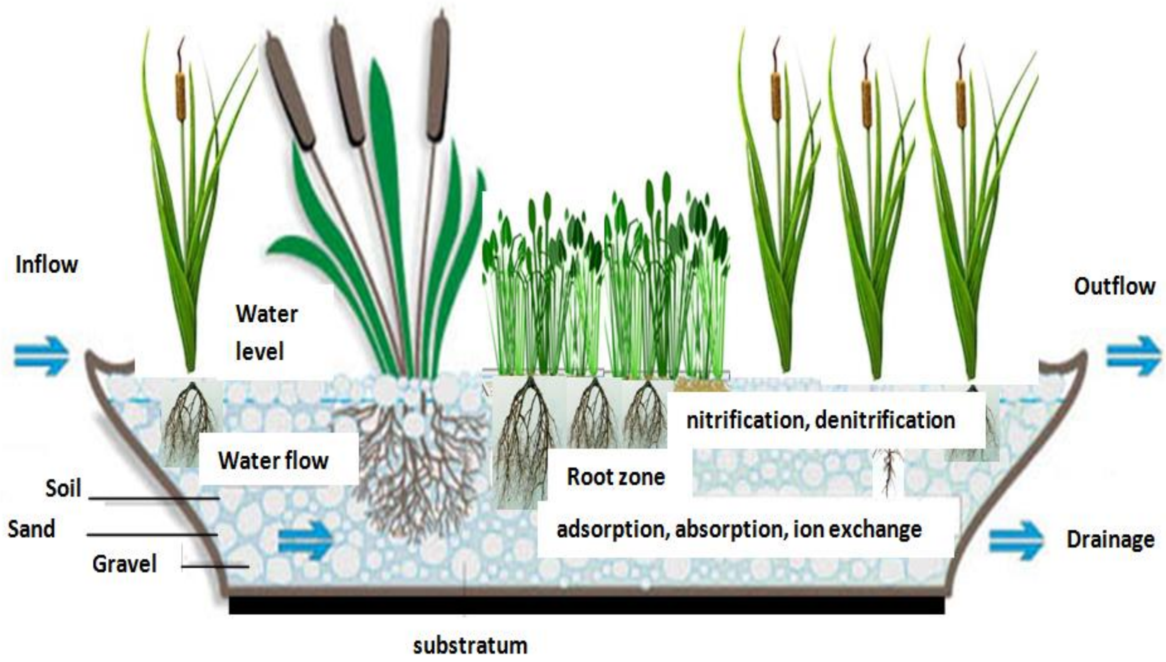
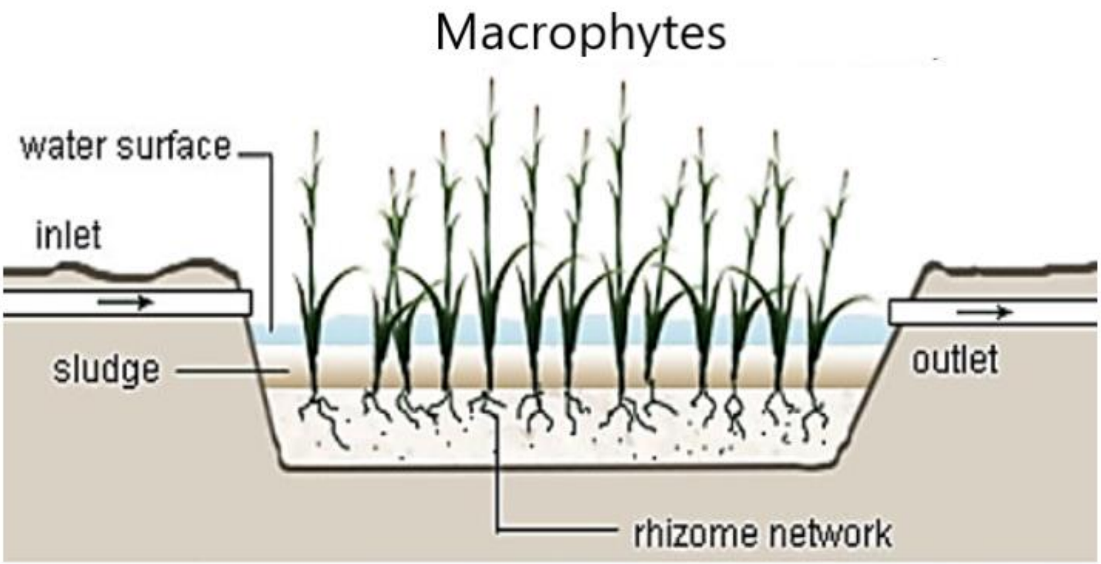


Fig. 1.3 Working model of a CWM unit having emergent macrophytes

1.3.1 Classification of CWMs

CWMs are categorized largely into three groups, – Surface Flow (SFCWs), Subsurface Flow (SSFCWs) and hybrid systems (Kumar and Dutta, 2019a). SSFCWs are further classified into HFCWs and VFCWs based on the flow pathway as given in Fig. 1.4 (Wang et al., 2018). They are also categorized based on the macrophytic growth - floating-leaved, free-floating, submerged and emergent macrophytes (Vymazal, 2010; Kumar and Dutta, 2019a). The SSFCWs have most wide applicability throughout the world and hybrid system also gained significant attention in contrast to others due to their high pollutants removal performance. They are setup to attain the advantages of the natural treatment wetlands under measured conditions. To develop well understanding about the removal processes linked with CWMs, numerous designs and working modes are existing to attain efficient DW treatment, for example single-stage alteration, multi-staged in sequence and/or together with another removal technologies (Singh et al., 2009; Melian et al., 2010; Kumari and Tripathi, 2014). Several scholars have contributed about the usage of CWMs for the treatment of wastewater worldwide (Vymazal and Březinová, 2015; Liu et al., 2015; Wu et al., 2015b; Kumar et al., 2020a). But there are comparatively fewer studies aimed to on-site wastewater treatment. There is an uncertainty about the choice of CWMs that offer more appealing results for DW in decentralized schemes (Kumar and Dutta, 2019b).



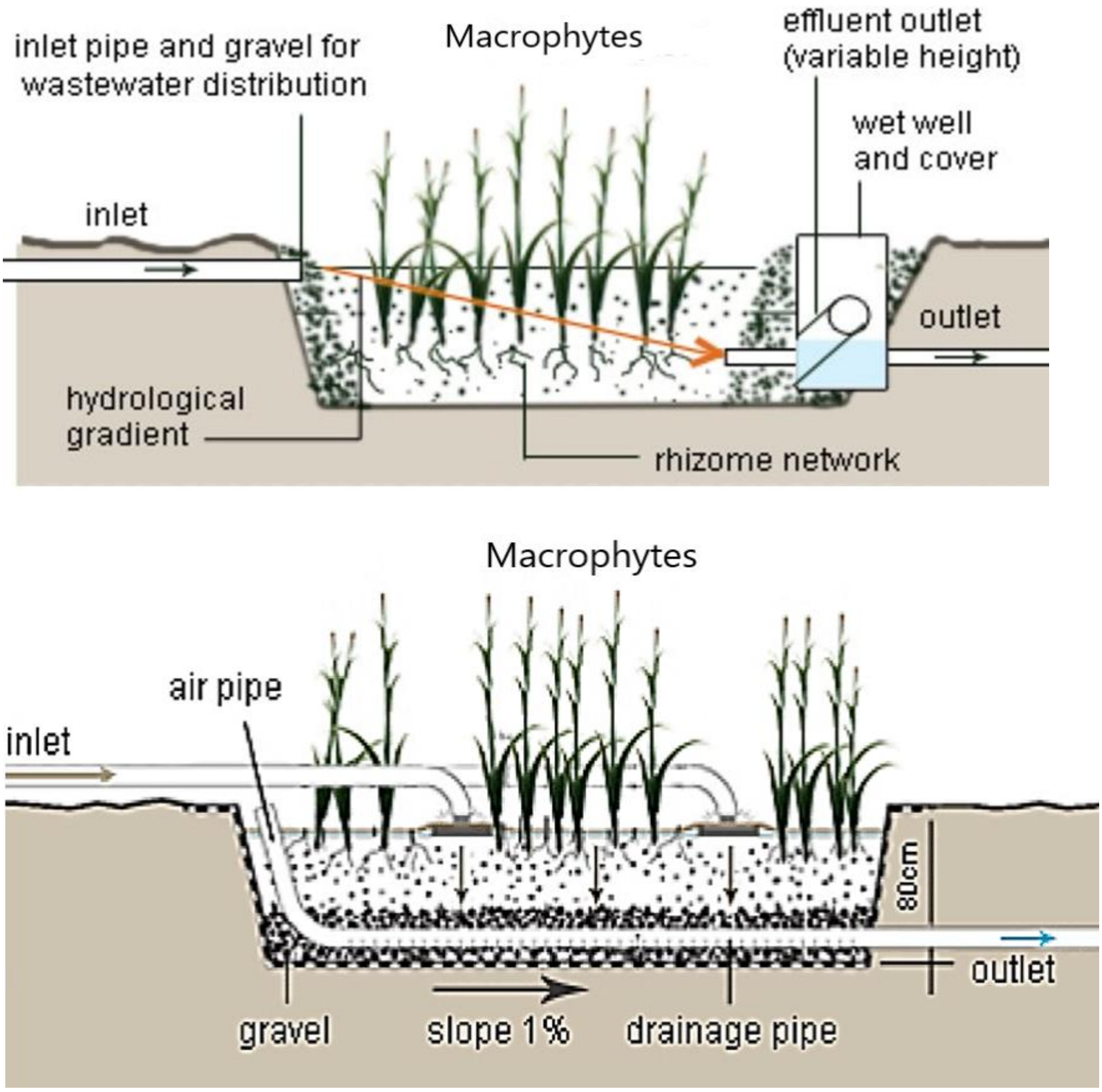


Fig. 1.4 Working models of Free water surface, Horizontal and Vertical flow CWs (adapted from Onyango et al., 2009)

These natural treatment wetland structures are measured as cost-effective and technically viable methods towards the treatment of various types of wastewaters in several continents:

- Considered as less expensive to create as compared to other treatment systems.
- Lower O&M expenditures and easy to maintain because they do not need extremely skilled operators.
- Withstand high fluctuations in flow and load of wastewater.
- Encourage biodiversity of the surroundings by increasing species diversity (Matamoros et al., 2012).

- Can be easily integrated with the natural and rural landscapes while offering habitat and aesthetic value as well as commercial worth from flowers and aquaculture (Llorens et al., 2009).

Several limitations are also associated with these wetlands treatment systems. Some of them are as follows:

- Usually, they require high land area as compared to other wastewater treatment technologies such as ASP (Kadlec, 2009).
- Poor design or maintenance may lead to extreme clogging problem, which might be affect the performance (Pedescoll et al., 2012).
- They do not work well with high discharge of wastewater.

1.4 Major components utilized in removal mechanisms

The major components employed in CWMs are macrophytes, substrate materials which may be artificial, industrial by-products or natural materials and microbial populations.

1.4.1 Wetland vegetation (Macrophytes)

Macrophytes are considered as prime vegetation for the treatment of wastewater in CWMs. They are basically categorized into four groups as explained below (Kumar and Dutta, 2019a). Macrophytes during the treatment process, transfer the oxygen, provide organics and substrate material for growth and attachment of microorganisms (Meng et al., 2014). They are also able to improve porosity and absorptivity of the substrate material and promote several bio-chemical reactions by acting as a catalyst (Yahiaoui et al., 2018). Globally, more than 150 macrophytic species have been tested for their potential towards efficient wastewater treatment in CWMs, though, only some of them are more frequently utilized. Emergent macrophytes are ideal agents as they show greater pollutant uptake and removal capability (Vymazal, 2013a). Macrophytes that are utilized in CWMs for the treatment of wastewater take part directly or indirectly in the removal processes such as uptake and assimilation of wastewater nutrients, surface insulation, provide oxygen and substrate material for the development of microorganisms, reduce wind velocity, regulate hydraulics and release exudates of various properties (Cui et al., 2010; Farzi et al., 2017; Hadad et al., 2018; Kumar and Dutta, 2019b). Several other functions such as physical filtration by roots, filter bed stabilization, de- clogging and insulation during cold winters are also facilitated by macrophytic vegetation within wetland systems (Valipour and Ahn, 2016). Macrophytic species such as *Phragmites spp.*, *Typha spp*., *Scirpus, Juncus, Canna indica* and *Pistia stratiotes* are most widely used in CWMs (Vymazal, 2013a) because of their great replication and flood-tolerant capabilities. They are mainly grouped into four categories based on their growth characteristics.

1.4.1.1 Emergent macrophytes

They habitually grow in water-logged conditions and are capable of thriving in 0.5 m or deeper water and have significant drought resistance. The main examples are *Phragmites spp*. (Poaceae), *Canna indica* (Cannaceae), *Juncus spp*. (Juncaceae), *Typha spp*. (Typhaceae), *Scirpus spp*. (Cyperaceae) and *Iris spp*. (Iridaceae) etc. Roots of these macrophytes transfer oxygen to rhizosphere for aerobic breakdown of contaminants (Vymazal, 2013b).

1.4.1.2 Floating leaved

They are totally rooted in the sediments/soil to 0.5–3.0 m water depth and have floating leaves on the water surface for examples *Nuphar lutea* and *Nymphaea odorata* etc.

1.4.1.3 Free floating

These are floating freely on the water surface and have significant potential towards the removal of nutrients, suspended solids and HMs via denitrification and uptake. For e.g., *Pistia stratiotes (Araceae), Eichhornia crassipes* (Pontederiaceae), *Salvinia natans* (Salviniaceae), *Marsilea quadrifolia* (Marsileaceae), *Trapa bispinosa* (Lythraceae), (Pontederiaceae), *Lemna minor* (Arecaceae) and *Azolla spp.* (Salviniaceae) etc.

1.4.1.4 Submerged macrophytes

These are totally submerged in water and grow well in an oxygen rich environment. They are mainly used for enhancing the wastewater quality after secondary treatment. For e.g., *Ceratophyllum demersum* (Ceratophyllaceae), *Hydrilla verticillata* (Hydrocharitaceae), *Myriophyllum spicatum* (Haloragaceae), *Vallisneria natans* (Hydrocharitaceae) and *Potamogeton crispus* (Potamogetonaceae).

1.4.2 Substrate materials

Presently in CWMs, most often used substrate materials are the natural, synthetic materials and/ or industrial by-products (Yan and Xu, 2014). Some commonly used substrate materials are listed in table 1.2.

Table 1.2 Common substrate materials that are regularly used in CWs

S. no.	Substrate type	Type of Wastewater	Reference
1	**Industrial by-products**		
	Fly ash	Municipal	Xu et al., 2006
	Slag	Domestic	Zuo et al., 2018
	Coal cinder		Ren et al., 2007
	Hollow brick crumbs		
	Alum sludge	Artificial	Babatunde et al., 2010
	Oil palm shell	Synthetic	Chong et al., 2013
2	**Natural material**		
	Gravel	Tannery	Lima et al., 2018
	Sand	Textile	Saeed and Sun, 2013
	Zeolite	Municipal	Bruch et al., 2011
	Clay	Tannery	Calheiros et al., 2008
	Limestone		Tao and Wang, 2009
	Organic wood mulch	Synthetic	
	Maerl		Saeed and Sun, 2012
	Shale		
	Peat	Domestic	
3	**Artificial material**		
	Activated carbon	Domestic	Ren et al., 2007
	Compost	Refinery	Saeed and Sun, 2012
	BOFS	Synthetic	Barca et al., 2014
	Lightweight aggregates	Synthetic	Lima et al., 2018
	Electro-oxidation	Hypereutrophic waters	Cao et al., 2014
	Rice straw		
	Light ceramsite		

The choice of substrate materials depends on the hydraulic permeability and its ability to absorb wastewater contaminants. This is due to the of reduced hydraulic conductivity may possess several consequences such as blockage of structures, clogging problem, lowering the adsorption process that ultimately affects the long-term removal efficiency of field-scale CWMs (Wang et al. 2010). Therefore, substrate materials must be selected on the basis of their ability to adhere/ absorb wastewater pollutants and their permeability. Permeability of the supportive material also affects the driving of water current within the CWMs. It is reported that substrate materials with reduced hydraulic conductivity affects the adsorption capacity critically (Wang et al., 2010). The long- term field scale operations of the CWMs for the treatment of wastewaters are known to be extremely impacted by the choice of substrate materials (Wang et al., 2010).

1.4.3 Microorganisms

The primary microbial population associated with wetland systems are bacteria, algae, yeasts, protozoa and fungi. Together, they take part in the breakdown of wastewater pollutants into less harmless constituents (Kumar and Dutta, 2019a). The efficient microbial populations are found attached with macrophytes roots, leaves and with substrates as biofilms (Faulwetter et al., 2009) that are developed by interactions with wastewater (Sleytr et al., 2009). Numerous earlier findings have recognized and characterized microorganisms in both full as well laboratory scale CWMs under defined conditions (Calheiros et al., 2009; Krasnits et al., 2009; Dong and Reddy, 2010; Zhang et al., 2010; Kumar et al., 2020b). Nevertheless, there is limited evidence of alteration in microbial populations and their diversity for long-term processes for DW treatment (Adrados et al., 2014). The organic matter present in wastewater within CWMs is utilized by microorganisms as substrate and expelled vitamins enhance the growth and activity of microorganisms recognized as ‘rhizosphere effect’. Microbes that are attached with substrate materials usually exist an extracellular polymeric substance (EPS) (Kumar and Dutta, 2019b). The rhizosphere region of CWMs has capability to provide add-on sites for the microorganism’s growth, release of root exudations and oxygen that assist in assessing the importance of microbial universe (Lv et al., 2017; Zhang et al., 2016).

1.5 Mechanisms of contaminants removal

The major pollutants removal mechanisms employed in CWMs are presented in table 1.3

Table 1.3 Major wastewater treatment mechanisms employed in CWMs (modified from Cooper et al., 1997; Mthembu et al., 2013)

Sr. No.	Wastewater Pollutants	Exclusion mechanism
1	Solids	Sedimentation, Filtration
2	Soluble organics	Aerobic and Anaerobic microbial breakdown
3	Nitrogen	Plant uptake, Ammonification, Nitrification, Denitrification, Ammonia volatilization and Matrix sorption etc.
4	Phosphorous	Plant uptake, Matrix sorption
5	Metals	Plant uptake, Adsorption, Cation exchange, Precipitation, Complexation, Microbial reduction and oxidation
6	Pathogens	Predation, Natural die-off, Sedimentation, Filtration Antibiotics released from Plants, UV irradiation

1.5.1 Removal of organic matter

Both anaerobic and aerobic microbial populations participate in the breakdown of organic matters depending on the availability of oxygen. For aerobic reactions, O_2 is provided by atmospheric dispersion, convection and macrophytic roots (Cooper et al., 1996). The pore space of substrate materials is accountable for anaerobic biodegradation. Suspended organic matter is excluded quickly by gravitational forces, however, soluble organic matter is eliminated by the growth of suspended or attached microorganisms.

1.5.1.1 Aerobic degradation

Aerobic chemoheterotrophs are main agents for such reactions due to their quicker metabolic activities as compared to chemoautotrophs. It is reported that the chemoheterotrophic bacteria break the organics in the presence of O_2 and release CO_2, NH_3 and other mixtures (Garcia et al., 2010). It is estimated that supply of oxygen at greater rate may improve the degradation rate by enhancing biochemical degradation (Vymazal and Kropfelova, 2009).

1.5.1.2 Anaerobic degradation

Degradation of organics anaerobically by heterotrophic bacteria comprises of mainly two processes viz. methanogenesis and fermentation. The methanogens break organics into CO_2 and CH_4 and develop new bacterial cells in methanogenesis. However, in fermentation process, acid-forming bacteria alter organic compounds into alcohols and acids as described by Kadlec (2018).

1.5.2 Removal of nitrogen

Wastewater that originates from communities contains mainly organic and inorganic nitrogen (Stefanakis et al., 2014). The main nitrogen exclusion routes associated with CWMs are categorized into two wide groups. These are novel and classical nitrogen elimination pathways (Saeed and Sun, 2012; Kumar and Dutta, 2019a).

1.5.2.1 Novel nitrogen exclusion pathways

Currently some more effective nitrogen elimination mechanisms are identified that comprise of partial nitrification-denitrification, Anammox and Canon etc. The description of these mechanisms is given below-

(a) Partial nitrification-denitrification

It requires 40% less organic matter and 25% less oxygen as compared to traditional mechanisms for the translation of NH_4-N to NO_2-N (Eq. 1) and NO_2-N to N_2 gas (Eq. 2) (Jianlong and Ning, 2004; Kumar and Dutta, 2019a).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

$$NO_2^- + 1/2CH_3OH + H^+ \rightarrow 1/2N_2 + 1/2CO_2 + 1.5H_2O \quad (2)$$

(b) Anammox

Anaerobic oxidation of ammonium is a newly discovered nitrogen elimination route in which NH_4^+ gets converted into N_2 gas with the help of *Planctomycetes* bacteria. This process is very crucial than other available nitrogen removal mechanisms in terms of less external carbon requirement. Additionally, the energy and oxygen inputs are also very low with greater nitrogen removal rate (Saeed and Sun, 2012).

(c) Canon

Complete autotrophic nitrite removal over nitrate utilized anammox and partial nitrification process both at the same time. The ammonium oxidizing bacteria together with anammox bacteria remove all available total nitrogen via autotrophic methods from a site. It is reported by Sun and Austin (2007) that this process alone is able to remove significant concentration of nitrogen.

1.5.2.1 Traditional nitrogen exclusion pathways

(a) Ammonification

Ammonification is the rate of conversion of organic nitrogen into ammonia and the conversion rate depends on temperature. A 10-degree Celsius increase in temperature doubles the rate of ammonification (Kadlec, 2018). The rate of ammonification is maximum in the top aerobic zone as compared to the lowermost anaerobic zone. The optimum pH value for the best performance lies between 6.5–8.5 (Saeed and Sun, 2012).

(b) Ammonia Volatilization

In this process, volatile ammonia gas is released into atmosphere via mass transfer (Eq. 3). It is a pH-sensitive process, a significant increase in pH (>9.3) can change NH_4^+ into NH_3 (Bialowiec et al., 2011).

$$NH_4^+ + OH^- \longrightarrow NH_3 + H_2O \qquad (3)$$

(c) Nitrification

It is a well-established nitrogen removal pathway for the wastewaters containing organic nitrogen. In the first step of reaction, NH_4^+–N is transformed into nitrite

(NO_2^--N) aerobically with the assistance of *Nitrosomonas, Chemolithotrophic, Nitrosospira* and *Nitrosococcus* bacteria (Eq. 4) and then NO_2^- is transformed into nitrate (NO_3^--N) in the presence of *Nitrobactor*, facultative *Chemolithotrophic* bacteria and *Nitrospira* (Eq. 5). However, the overall result of this process has been given in Eq. 6.

$$NH_4^+ + 1.5O_2 \xrightarrow{\text{Nitrosomonas}} H^+ + H_2O + NO_2^- \qquad (4)$$

$$NO_2^- + 0.5O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \qquad (5)$$

The final result is given below:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O \qquad (6)$$

(d) Denitrification

In this process, the nitrate gets converted into nitrogen gas (Elfanssi et al., 2017). Denitrification process is considered as main nitrogen removal pathway as it releases gaseous nitrogen into the atmosphere. Several facultative bacteria such as; *Bacillus, Pseudomonas, Spirillum, Enterobacter* and *Micrococcus* (Kadlec and Knight, 1996) take part in this reaction. The overall denitrification reaction and their products has been given by USEPA (1993) and Kadlec and Knight (1996) in Equation (7) and (8) respectively.

$$NO_3^- + 0.833\ CH_3OH \rightarrow 0.5N_2 + 0.833\ CO_2 + 1.16\ 7H_2O + OH^- \qquad (7)$$

$$NO_3^- + 1.08\ CH_3OH + 0.24\ H_2CO_3 \rightarrow 0.056C_5H_7NO_2 + 0.47N_2 + 1.68H_2O + HCO_3^- \qquad (8)$$

(e) Plant uptake

Macrophytes uptake nitrogen mainly in the form of nitrates and ammonium as source of nutrients for their growth. Existence of macrophytes is crucial for advancing removal efficacy, as explained below -

(a) Providing surface and oxygen for the growth and development of microbial populations within root zone (Cui et al., 2010).

(b) Providing carbon from roots and optimizing the denitrification reaction (Wang et al., 2012).

The concentration of nitrogen taken up by macrophytes differs depending upon local environmental conditions, loading rates, system design, type of wastewater. It is observed that the role of macrophytes in nitrogen removal varied from 0.5 – 40.0% (Drizo et al., 1997; Bialowiec et al., 2011). It is described earlier that the macrophytes contributes upto 9% in the elimination of nitrogen (Meers et al., 2008).

(f) Adsorption

Adsorption of nitrogen into the substrate materials mainly proceeds via NH_3 (Tsihrintzis, 2017). The process is greatly regulated by the substrate material that is used to enhance the cation exchange capacity. Substrate materials with superior CEC have been preferred because of their greater adsorption capacity (Saeed and Sun, 2012).

1.5.3 Total phosphorus removal

The mixture of natural and inert phosphate is existing in a wastewater drain predominately in the form of orthophosphates (PO_4^{3-}). The phosphate removal efficacy of CWMs relies on the available form of phosphate, local ecological conditions, loading rates and type of macrophytes etc. (USEPA, 2000). It is observed that reduced flow velocity with higher water depth enhances the phosphorus removal (Guo et al., 2017). However, the exclusion rate is controlled by phosphate immobilization, temperature and the adherence ability of substrate materials. Dissolved form of phosphorus is taken up by available macrophytic species and also adhered on the surface of substrates in the presence of cations like Mg, Ca, Fe and Al in excess. This process involves allocation of OH^- ions and H_2O on the surface of Al and Fe oxides by phosphate through ligand exchange reactions. However, the rate of phosphorus removal varies significantly depending on the adsorbent material used as substrate (Vymazal, 2010). Various specialized substrate materials have been identified recently to improve the performance of CWMs such as BOFS, slag, zeolite, sandstone, bauxite, dolomite EAF etc. (Okochi and McMartin, 2011; Barca et al., 2014; Stefanakis et al., 2014). Various forms of phosphorus such as inorganic, organic, insoluble and dissolved phosphate is taken up by macrophytes after conversion into soluble state (Choudhary et al., 2011). Macrophytes possess inferior phosphorus uptake ability as compared to nitrogen due to the-

a. Insoluble phosphate precipitated in the presence of Al, Ca and Fe ions under aerobic setting.
b. Oxides and hydroxides of Al and Fe, organic peat, and clay have shared phosphate adsorption.
c. Binding with organics via assimilation by algae, bacteria and macrophytes.

Several artificial substrate materials have been also identified to improve adsorption capability which includes magnetic iron oxide nanoparticle (MION), zirconium oxide nanoparticle (ZON) and granular activated carbon with iron oxide coating (Fe-GAC) etc. Due to manufacturing difficulties and generation of secondary pollutants during the production and high-cost inputs, use of such materials is very scarce (Park et al., 2017).

1.5.4 Removal of heavy metals

Presence of trace metals in the wastewater possess significant negative impacts on the aquatic biodiversity of the aquatic ecosystem (Parnian et al., 2016). Therefore, exclusion of these toxic metals from wastewater is essential before the discharge in fresh waterbodies. Removal of these heavy metals from wastewater utilize numerous methods in which adsorption, ion-exchange, reverse-osmosis and electrodialysis are more frequently used. Due to high-cost involvement, energy-intensive and metal specific nature, these are not feasible especially in developing countries. In addition to1 these, macrophytes in the CWM systems are identified with great potential for trace metals accumulation in their roots and aerial parts (Mishra and Tripathi, 2008). Remediation of DW using CWM technology comprises several mechanisms such as sedimentation, filtration, precipitation, macrophyte uptake, cation exchange, adsorption, microbial oxidation and reduction and complexation. Numerous abiotic and biotic and ecological aspects like DO, temperature and pH have significant impacts on the removal capacity of trace metals within CWMs (Xing et al., 2013).

1.6 Sustainability of CWMs

A successful working model of CWMs for the treatment of DW comprises of the appropriate design at suitable site with effective macrophytic population and substrate materials. Design of CWMs must be according to natural cycles to lessen the dependency on external forces. Designing on the basis of predominant environmental conditions, accessibility of land and geology is mostly performed. Source of additional organics externally and O_2 via aeration, bioaugmentation of specific microorganisms, optimization of HLR and HRT water depth, and regular harvesting of macrophytes improve the performance and sustainability of CWMs (Fig. 1.5) (Kadlec and Wallace, 2009; Kumar and Dutta, 2019b). Several environmental factors such as pH, DO and temperature are critical for the proper and effective working of CWMs. The performance of CWMs drops significantly when these ecological parameters are not suitably maintained (Kadlec and Wallace, 2008; Wu et al., 2015).

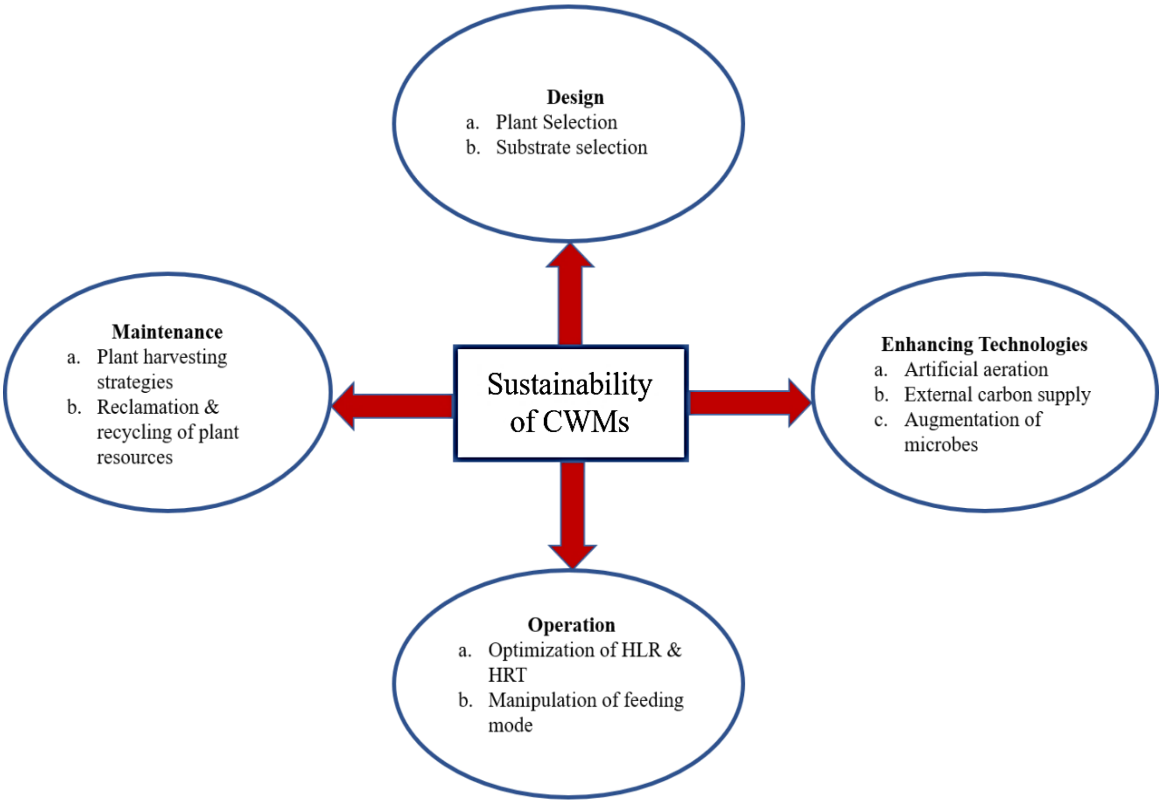


Fig. 1.5 Several aspects of CWMs associated with smooth and sustainable functioning

Objectives

1. **To evaluate the treatment efficiency of constructed wetland microcosms and assess the key factors affecting the performance**
    - Removal efficiency of selected heavy metals and other wastewater parameters by different CWM units at three retention times.
    - Removal kinetics of heavy metals based on the decay constants
    - Impacts of pH, DO and temperature and other parameters such as solar intensity, humidity on removal efficacy of CWMs
2. **To study the interspecific competition and their impacts on the growth of macrophytes and pollutants removal within constructed wetland microcosms**
    - Interspecific competition among different macrophytes and their relative growth rate (RGR)
    - Impact of interspecific competition on the growth and nutrient uptake of macrophytes
    - Study of several growth-related parameters at different time intervals
3. **To assess the activity of different enzymes (Dehydrogenase, urease, phosphatase, fluorescein diacetate hydrolysis and microbial biomass carbon) and their impacts on the performance of constructed wetland microcosms**
    - Vertical and temporal variation in the activity of different enzymes at two soil substrate depths (0-10 and 10-15 cm respectively)
    - Correlation among activity of enzymes and contaminants removal at three retention times
4. **To compare the acceptability of constructed wetland microcosms with respect to other available treatment techniques such as Upflow Anaerobic Sludge Blanket Technology (UASB) and Fluidized Aerobic Bed Reactor Technology (FAB).**
    - Compared the acceptability of CWM with these USAB and FAB based reactors by the data collected from two STPs for two years working in the Lucknow
    - Integration of CWMs with these wastewater treatment technologies as final polishing system and further reuse of treated water

Chapter 2
Review of Literature

2.1 Introduction

Water is a vital resource for the existence of all the living creatures on the earth. However, this elixir resource is gradually being threatened by increasing demand for agricultural and domestic purposes due to rapid population and economic growth. Amongst the other environmental challenges in India, the water scarcity is a major cause of concern. The main obstacles towards the better management of the water resources in India are inappropriate management of runoffs, lack of adequate treatment capacity within urban settlements, unplanned industrial establishments, uneven pattern of rainfall, irregular geographic dispersal of water resources, overextraction and contamination of groundwater. Due to centralized schemes and inadequate local budgets to develop proper drainage networks, the majority of DW are discharged directly into nearby water streams resulting into serious contamination (Kumar and Dutta, 2019b; Li et al., 2021). These practices are very common in developing countries particularly in decentralized and distant settlements (Yang et al., 2018). Numerous traditional wastewater technologies such as activated sludge process (ASP), membrane bioreactors, UASB, FAB and membrane separation etc. are utilized in several metropolitan cities for managing their DW. Because of high-cost involvement, high loads of wastewater and their low treatment efficiency, the application of these technologies is limited at large scale (Zhang et al., 2020). Several environmental factors such as DO, pH and temperature are vital for the effective working of CWMs. Working parameters such as selection of macrophytic species and substrate, suitable design at appropriate site, artificial aeration, plant harvesting strategies, C/N ratios, effluent recirculation, HLR, HRT, bioaugmentation of microbial populations, effect of feeding mode and supply of external carbon are very important. Numerous environmental and working parameters are described below-

2.2 Environmental factors

2.2.1 pH

Several reactions and mechanisms within CWMs are governed by the pH of wastewater along with solubility of gases and solids, cation exchange and biotic conversion (Niveditha 2019). It regulates numerous biological reactions and supports ionization of the complexes. The growth of macrophytes and action of heterotrophic and nitrifying bacteria requires optimal pH. However, the existence of macrophytes within CWs also controls the pH (Ilyas and van Hullebusch, 2020). It is reported by Sun and Austin, (2007) that the biotic reactions are seriously influenced by the wastewater pH within treatment wetlands. The best suited pH essential for the action of *Nitrosomonas* ranges from 7.9 to 8.2, however, the range from 7.2–7.6 is found suitable for the action of *Nitrobacter*. The activity of denitrifying bacteria is found significant at pH 6.5–7.5 as reported earlier (Huang et al., 2019). Several previous researches also identified that these bacteria achieved good results at pH range of 7.0 - 7.5 (Kraiem et al., 2019; Fu et al., 2020; Wei et al., 2020). A significant drop in pH is also observed during the nitrification reactions. However, the activity of methanogens is more at pH ranging from 6.5-7.5. It is well established that a slight variation in pH may reduce the growth and action of methanogens consequently leading to the development of odorous constituents as explained by Saeed and Sun, (2012).

2.2.2 Temperature

Temperature plays critical role in the efficient working of CWMs by encouraging microbial action to enhance the degradation of contaminants (Wang et al., 2016). Numerous contaminants exclusion processes are directly linked with temperature; therefore, a substantial modification in temperature can distress the removal rate (Zhang et al., 2018). The optimum temperature for the nitrification reaction within wetlands system varied from 16.5 – 32^{o}C. However, a rise above 40^{o}C or reduction upto 5^{o}C can alter the performance of nitrifying bacteria considerably (Shi et al., 2018). The process of nitrification influenced negatively once the temperature lowers than 10°C and the situation became very critical when it falls down to 6°C (Chang et al., 2012). Likewise, denitrification reaction is also become slow when temperature drops lower than 5°C however, it acquires higher removal rate when temperature ranges from 20 - 25^{o}C (Shi et al., 2018). Elimination of phosphorus also possess a great dependance on temperature. A considerable rise in temperature enhances the growth and action of phosphate-degrading bacteria (Wang et al. 2016). All types of wetlands system expressed higher heavy metals removal capability in summer as compared to winter due to enhanced macrophytic metabolism and microbial action. Several useful and necessary design adjustments in conventional CWMs have been recommended to lower down the temperature effects. For example, utilization of mixed substrate as packed media with sawdust layer, is identified as an ‘insulating layer’ to treat DW (Wu et al. (2011). These changes within substrate bed exhibited a small decrease in removal efficacies by sustaining optimum temperature throughout the winters. Different seasons throughout the year pose major challenge on the performance of CWs. The mean concentrations of total nitrogen (TN) in effluent of CWs have been recorded lower throughout the summer that may be because of higher plant uptake during summer seasons. It is also reported that the involvement of different macrophytes towards the elimination of nitrogen is only around 0.5 - 40% (Seed and Sun, 2012; Kumar and Dutta, 2019b). Degradation of pollutants via microbial activity within substrate materials generate adequate heat to protect the exterior layers from freezing. The majority of the macrophytes, such as *Phragmites spp., Typha spp., Canna generalis, Acorus calamus* and *Juncus effusus* in winter season show inactivity eventually lessening the growth (Fan et al., 2016). Besides, the activity and range of microbes also get suppressed (Wu et al., 2015).

2.2.3 Availability of dissolved oxygen (DO)

Dissolved oxygen is one of the most significant factors which can alter contaminant's elimination and microbial activity in CWs (Liu et al., 2015). The major disadvantages of traditional CWs are inadequate and unsuitable oxygen supply and circulation. However, it regulates several biodegradation mechanisms of organics and nutrients inside the matrix in substrate bed (Vymazal and Kropfelova, 2009). To attain high organics and nitrogen elimination rate, proper oxygen and carbon source supply are necessary. The removal of nitrogen has been enhanced significantly to the range of 76.9 - 86.0% and 72.3 - 89.1% as TN and NH_4^+-N respectively along with aeration (Zhang et al., 2010). A study carried out by Maltais-Landry et al. (2009) on CWMs planted with emergent macrophytes with intermittent aeration exhibited the complementary role to smoothen the TN removal. The occurrence of emergent macrophytes with aeration enhanced removal efficiency of several contaminants present in the DW. Supply of external oxygen via aeration are crucial to withhold aerobic and anaerobic regions, mainly when simultaneous nitrification-denitrification is required. Exclusion of nitrogen through CWs based on DO profile of effluent might not continuously demonstrate the actual description of the microbial responses (Saeed et al., 2012). It is also reported that higher DO concentrations in the effluents from CWMs does not always express the aerobic conditions. Anaerobic and aerobic areas are simultaneously existing (Bakhshoodeh et al., 2020).

2.3 Working/ Operational parameters

2.3.1 Choice of macrophytes and substrate materials for better performance of CWMs

Almost all types of CWs encourage aquatic life by promoting their growth and development. The macrophytes provide substrate for adherence sites to microbial populations by forming microhabitats and by decreasing the water velocities. The death of macrophytes and their parts offers supplementary nutrients and carbon essential for microorganisms' expansion for further action. Macrophytes like *Phragmites spp.* and *Typha spp.* have advanced pollutants exclusion efficacy (Kumar and Dutta 2019b). These are regularly used in the CWMs for the treatment of wastewater throughout the countries including Asia and Europe. The main roles of macrophytes planted in CWMs for the treatment of DW are: (a) offer litter for microbial growth (b) reduce water velocities for the enhancement in contact time (c) provide adherence sites for microbial growth and development through root arrangements and stems (d) help in settling down of suspended materials (e) transfer gases and develop oxygenated microsites within substrate bed and build wastewater contaminants including toxic metals in their tissues (Kumar et al., 2020c). Approximately 150 macrophytes have been identified for their successful work in CWMs globally, yet, only some of them are in regular use. It is well established that emergent macrophytes are perfect candidature for the efficient functioning of CWMs treating DW (Vymazal, 2013a). Growth characteristics and nutrient uptake capacity of various regularly used macrophytes within CWMs have been provided in table 2.1.

The selection of substrate materials strongly depends on its capability to adsorb/absorb wastewater pollutants and their permeability. Substrate materials with lower hydraulic conductivity cause clogging of the system and reduce the adsorption process consequently, the removal efficiency and long-term operations of CWMs have been affected seriously (Wang et al., 2010). Several studies have been done throughout the world on selecting the correct substrate materials (Saeed and Sun, 2012; Wu et al., 2015). Several synthetic, natural materials and industrial by-products are frequently used as substrate materials in all types of CWMs (Yan and Xu, 2014; Kumar et al., 2020a). The substrate materials mainly comprise of artificial media, industrial by-products and natural materials (Saeed and Sun, 2012; Kumar and Dutta, 2019b). It is also estimated that the natural substrate materials are less appropriate as compared to synthetic materials and industrial by products for long term operations due to their lower hydraulic permeability (Vymazal, 2007).

Table 2.1 Growth characteristics of various regularly used macrophytes within CWMs

Type	Macrophytes	Optimal pH	Optimal temperature (ºc)	Maximum water depth (inch)	Growth	Root penetration (cm)	Drought Resistance	Nutrient uptake abilities ($Kg\ ha^{-1} yr^{-1}$) Nitrogen	Phosphorous
Emergent	*Typha sp.* (*Cattail)*	4 - 10	10-30	12-18	Rapid	75	Possible	1000	180
	Phragmites sp. (Common Reed)	3.7 - 8	12-23	3	Very rapid	60	High	2500	120
	*Juncus sp. (*Rush*)*	5 - 7.5	16-26	3	Rapid	25	Moderate	-	-
	*Scirpus sp. (*Bulrush*)*	4 - 9	16-27	12	Moderate to rapid	75	Moderate	-	-
Free-floating	*Pistia stratiotes (Water lettuce)*	6 - 6.8	15-35	-	Very rapid	80	No	900	40
	Lemna sp. (Duckweed)	6.5 - 7.5	6-33	19	Very rapid	2	No	-	-
	Eichhornia crassipes (Water hyacinth)	6.5 - 7.5	12-35	-	Very rapid	100	No	2400	350

Source: author`s own elaboration

2.3.2 Effects of C/N ratios

The optimum C/N ratio for maximum removal of nitrogen lies between 2.5 to 5 (Chen et al., 2020). They also observed that the ideal value of C/N ratio to create equilibrium state among denitrification and nitrification is 10. The performance of these nitrogen removal processes gets transformed when C/N ratio becomes higher. The performance of CWMs for nitrogen removal also depends on the background concentration of contaminants (Saeed and Sun, 2012). Supply of additional carbon is essential for the denitrification reactions, mainly when the C/N ratio drops below 3 as observed by Stefanakis et al. (2014). A study on three dissimilar C/N ratios in VFCW stated that the maximum elimination of TN was observed at C/N 2.5 (Zhao et al., 2010). Similarly, Chen et al. (2020) identified that the maximum elimination of TN was found at 5 and 10 C/N ratio for HFCW. From these previous findings, it may be concluded that the optimal value of C/N ratios varied significantly based on the types of CWMs, wastewater characteristics, choice of macrophytes and pollutants to be removed. However, use of organic substrate (organic wood-mulch and rice husk) as filter material can supply organic matter to smoothen the heterotrophic exclusion of NO_3^--N (Tee et al., 2012).

2.3.3 Selection of suitable site and design for CWMs

Selection of appropriate site for proper functioning of CWMs can save considerable expenses. Prior to design, it must be essential to study the availability of the land, landscape settings, land use, soil, ecological resources and the possible impacts on neighbors. A suitable site for setting up CWM system may be considered as: a) located near the source of wastewater b) designed above the water table, away from flood-prone area c) positioned at sloppy areas to use gravitational forces to move wastewater d) containing compact soil to prevent from leakage to water table e) unavailability of threatened and endangered species in that area f) large space to handle present requirements and upcoming adjustments and amendments. However, appropriate design of CWMs are systematic and scientific efforts to mimic the role of natural wetlands. Various studies have been conducted to optimize the wetland procedures to enhance the water quality. Mitsch (1992) recommends following guidelines to build an operative CWM system, these are : a) Simple in design b) minimum maintenance requirements c) use of natural services like gravity flow d) design against the extreme meteorological events such as storm, flood and drought e) design must be based on dominant ecological settings d) integration with natural landscape of the location f) must be avoided from over-engineering with simple rectangular sections g) sufficient time requirement for smooth functioning as CWMs do not fundamentally act instantly, h) design must be for the functioning, not for form. Successful examples of CWMs for the treatment of different types of wastewaters from various parts of the world have been provided in table 2.2.

Table 2.2 Successful examples of CWMs worked/ working for the treatment of different types of wastewaters throughout the world

Wastewater	(Dimension) (L x W x D)	Macrophytes	Plant Density	HLR	HRT	Study area/ Country	Reference
Municipal	Area 185.5 m^2 and depth 0.85 m	*C. papyrus*	NA	0.18, 0.10 and 0.07 m^3/m^2. d	1.8, 3.2 and 4.7 d	Egypt	Abou-Elela et al., 2017
Urban	1 = 1.0 * 1.5 * 1.3	*P. australis*	NA	0.37 m d^{-1}	21 hrs.	Spain	Avila et al., 2017
Domestic	0.95 * 1.15 m	*H. psittacorum*	NA	0.15 m d^{-1},	NA	Colombia	Bohórquez et al., 2016
	A = 200 m^2 B = 360 m^2	*P. australis* *T. latifolia*	4 m^2	0.46 m^3/m^2/d 0.1 m^3/m^2/d	0.7 1.2 d	UK	Butterworth et al., 2016
Secondary	0.66 m^2	*C. articulatus*	33 m^2	0.46 md^1	3 d	Colombia	Caselles-Osorio et al., 2017
Domestic	1.0 * 0.6 * 0.4 m	*C. ligularis* *E. colona*	38 m^2	0.06 m^3/d	2.3 d	Colombia	Casierra-Martínez et al., 2017
	13* 10 * 0.9 m	*P. australis*	4 m^2	0.5 and 0.75 m^3/m^2. d	NA	Morocco	Elfanssi et al., 2017
	8.4 * 5.4 *0.45 m	*J. acutus, T. parviflora, L. monopetalum and S. perrenis,*	1 m^2	53 mm/d	3.48 d	Greece	Fountoulakis et al., 2017a
	1.20 * 0.90 * 0.95 m	*J. acutus A. halimus*, and *S. perennis*	9 m^2	95 mm/d	NA	Greece	Fountoulakis et al., 2017b
Synthetic	43 * 32 * 7 cm	*P. arundinacea, R. japonicas, O. hookeri, and R. carnea*	12 m^2	NA	10 d	China	Geng et al., 2017
Municipal	14 ha	*Salix spp, H. vulgaris.* and *Carex sp.*	NA	NA	11–14 d	Canada	Hayward et al., 2014
Municipal and	7.5 * 3.0 * 0.6 m	*C. papyrus*	NA	NA	NA	Kenya	Mburu et al., 2013

Wastewater	**(Dimension) (L x W x D)**	**Macrophytes**	**Plant Density**	**HLR**	**HRT**	**Study area/ Country**	**Reference**
secondary							
Domestic	30.0 * 6.0 *1.0 m	*C. generalis*	4 - 5 m^2	$0.02 m^3 s^{-1}$	3hrs	India	Ojoawo et al., 2015
Urban sewage	7.8 * 6.65 * 1.8 m	*P. australis, T. latifolia,* and *C. esculenta*	NA	NA	12 - 36 hrs.	India	Rai et al., 2015
Municipal	2.0 * 2.0 * 10 - 60 cm	*L. Perenne*	10 cm^2	0.0375 $m^3/m^2 \cdot d$	6 d	China	Ren et al., 2016
Domestic	11.8 * 8.6 * 1.2 m	*T. latifolia L* and *S. tabernaemontani*	NA	16.62 m^3/d	NA	Canada	Rozema et al., 2016
Synthetic	0.5 * 0.4 * 0.3 m	*E. crassipes*	NA	NA	2 d	Brazil	Lima et al., 2018
Domestic	10 * 3 * 1.5 m	*A. conyzoides, T. latifolia, P. Stratiotes* and *C. indica,*	NA	10 cm/d	5 d	India	Tilak et al., 2017
	Height 65 cm and diameter 20 cm	*P. australis*	8 / system	NA	72 hrs.	China	Wu et al., 2016
Secondary	120 - 320 m wide and 15000 m long	*T. orientalis, P. australis, Z. latifolia, P. crispus, N. nucifera, L. minor, N. tetragona, and E. crassipes*	NA	3.5 cm d^{-1}	7 d	China	Wu et al., 2018
Synthetic and Secondary	1.2 * 0.6 * 0.6 m	*T. angustifolia*	14-15 m^2	5.6 cm/d	4 d	Singapore	Zhang et al., 2012
Synthetic	51 * 38 * 18 cm	*J. effusus L, R. japonica, P. arundinacea L.* and *O. javanica.*	12 m^2	NA	NA	China	Zhao et al., 2016
Domestic	65 cm in height and 20 cm in diameter	*O. javanica*	NA	NA	NA	China	Zhou et al., 2018

Source: authors’ own elaborations NA*= Not Available

2.3.4 HLR and HRT

These are the leading factors for the efficient working of a CWM system. A slight change in these parameters can affect the performance of the systems significantly. HRT is expressed as interaction time of pollutants with substrate materials and macrophytes. It is well established that larger HRT favors the elimination efficacy for all contaminants (Stottmeister et al., 2003). An extended time period allows widespread interface amongst contaminants and several constituents of the CWMs. However, with shorter HRTs, wastewater passes out quickly and ultimately lessens the contact time with microorganisms and macrophytic roots and finally reduce the overall performance (Tuncsiper et al., 2015). It is also observed that several biogeochemical processes and microbial inhabitants and their arrangement are greatly influenced by HRT (Ranieri et al., 2013). Extended HRTs usually need extra land area and more investment. Thus, forthcoming studies are required to assess the influence of dissimilar HRTs on the removal efficacies of CWMs. However, HLR may be defined as volume of influent applied in the treatment unit per time period. HLR simplifies the faster drive of the wastewater via substrate materials and reduces the contact time (Kumar et al., 2020c). Extensive research on working of 10 VFCW for synthetic wastewater treatment with different HLRs have been conducted by Stefanakis and Tsihrintzis (2012) for three years. They applied three dissimilar HLRs (0.19, 0.26 and 0.44 m/d respectively) for three consecutive years. The outcome of this study revealed that with increasing HLR, the removal performance also increased significantly. Cui et al. (2010) investigated that increase in HLR from 7 to 21 cm/d, reduce the elimination efficiency from 65 to 60% and 30 to 20% of TN and NH_4^{+}-N respectively from DW in VFCWs.

2.3.5 Influent feed mode and effluent recirculation

Selection of suitable feed mode is necessary as it allows the mixing of effluent inside the substrate bed. Presently, numerous types of feed modes have been described like batch step, intermittent and continuous feed and tidal flow modes to advance the treatment efficacy. Laber et al. (1997) examine the effect of irregular loading on the removal efficacy of VFCWs to improve the nitrogen conversion from domestic sewage. The noteworthy result of this work was the advanced denitrification rate after the external carbon supply. On the contrary, the rate of denitrification becomes slow in continuous loading mode. The impacts of intermittent and continuous feeding have been also estimated by Caselles-Osorio and García (2007) for HFCW. They found that the intermittent feeding of influent significantly enhanced the removal efficacy of NH_4^{+}-N. For that, they hypothesized two possible elucidations, such as macrophytes provided with maximum DO and associated flushes throughout intermittent loading encourage more turbulence inside the substrate bed. Due to that wastewater transfers into aerobic and anaerobic zones simultaneously. Several wide-ranging alterations in feeding mode have been functional for the elimination of nitrogen and organics via efficient utilization of aerobic settings inside the substrate bed. The VFCWs with tidal flow feed mode is considered as an appropriate candidate (Wu et al., 2018).

Recirculation of influent within a CWM system upgrades the exclusion of nitrogen mainly in lower DO conditions (Lavrova and Koumanova, 2010). The denitrification rate is also enhanced because the time of interaction among wastewater and biofilms gets improved by the additional organics supply (Zhao et al., 2004). Numerous findings report many recirculation ratios to gain higher nitrogen removal. A hybrid CWs system to treat DW was designed by Ayaz et al. (2012). They fix the recirculation ratio at 1:1-2:1 and gain noteworthy rise in the exclusion of TN as compared to without recirculation. Similar results were also obtained by Lavrova and Koumanova (2010) for the DW treatment in VFCW system. The basic principle of effluent recirculation is to dilute the receiving wastewater therefore, the removal capacity is ultimately improved. Recently, an improved version of VFCW as RVFW (recirculating vertical flow wetland) has been established (Sklarz et al., 2009). This system provides nonstop recirculation of effluent to the rhizosphere till the optimal discharge quality of effluent is attained. Implementation of this RVFW system for DW management is more useful as it (a) provides extensive aeration and can dilute the wastewater (b) conservation of microbial biomass by substrate bed saturation. However, implementation of this system showed limitations for areas having high continuous loading and it also involves high energy.

2.3.6 Appropriate harvesting strategies of macrophytes

The harvesting of macrophytic biomass is being done typically to remove pollutants that are accumulated in vegetative fragments. Harvesting of macrophytes periodically is considered as great option for the suitable working of CWMs for the treatment of

DW (Ranieri and Gikas, 2014). The optimal time period for harvesting is evaluated via interface among total biomass and nutrient concentrations. It is reported that harvesting of macrophytes during the summer months delays the succeeding growth of planted macrophytes and also can disrupt the movement of nutrients inside the parts of macrophytes by ROL (radial oxygen loss) (Wang et al., 2015a). It is also defined that harvesting in the late autumn can have adverse effects on the NH_4^+-N removal. Additionally, the working mechanisms of macrophytes are different in several seasons. However, the impact of summer harvesting on the performance of CWs and microbial diversity is still not well explained. It is observed that harvesting can also lead to the nitrogen removal (Jinadasa et al., 2008). Kadlec et al. (2000) reported that harvesting can eliminate nitrogen from 0.27 to 0.68 g/m^2/d. It is reported that 120 kg/ha of nitrogen was removed by summer harvesting (Borin et al., 2001). In summer months, the growth of macrophytes is more vigorous therefore, the uptake capacity of contaminants is also higher as compared to winter seasons (Greenway, 2005).

2.3.7 Artificial aeration and bioaugmentation of microbes

It is reported that artificial aeration improves the removal capacity for organics and nutrients significantly (Wang et al., 2015b). It improves bacterial nitrification through offering optimum environments for their activity, however, removal of phosphorus is enhanced via oxidation of Fe^{2+} to Fe^{3+} as described by Li et al. (2014). Furthermore, aeration also improves the working life of CWMs by inhibition of blockage through mineralization of solids and managing the inconsistent loads (Butterworth et al., 2016). It is described that intermittent aeration together with sludge-ceramsite as a substrate significantly enhances the nitrogen elimination (Wu et al., 2016). It is also evaluated that the intermittent aeration is more effective than continuous as it brings more nitrite and ammonia -oxidizing bacteria (NOB and AOB). Numerous studies introduced especially adapted bacterial communities with anammox bacteria within modified substrate to enhance the elimination of nitrogenous compounds (Zou et al., 2009; Wang et al., 2011; Zaytsev et al., 2011). The bioaugmentation of new specific bacterial populations reformed the microbial structure via varying the species richness and developing new microbial cosmos (Zhao et al., 2016). The microorganisms present in the substrate material of wetlands having less contaminated wastewater may transform more phosphorus as compared to wastewater loaded with high pollutants. It is reported that the *Paenibacillus sp.* of bacteria own the maximum removal capability of TP (Hou et al., 2011). Therefore, bioaugmentation of specific microbial populations within the substrate material in CWMs can offer a low cost and best alternative especially for colder regions. However, there is limited information on the viability and removal efficacy of such bioaugmented CWMs in colder areas (Wang et al., 2012).

2.4 Major microorganisms associated with CWMs and their roles in removal process

Identification and characterization of microorganisms' communities may provide crucial information towards the understanding of their role in removal process (Zhong et al., 2015). It is reported that the microorganisms need about 75–100 d to make their communities within CWMs having sand as substrate material (Weber and Legge, 2011), Whereas, the denitrification and ammonium- oxidizing bacteria need 75 and 95 d respectively for their growth and expansion (Wang et al., 2016). PCR-DGGE is a useful instrument for assessing the diversity of microorganisms (Ibekwe et al., 2003). Muyzer et al. (1993) applied this technique for the first time followed by Adrados et al. (2014) to assess the structure of microbial populations. Additionally, the microbial biomass carbon (MBC) is also a significant mechanism used for the study of microbial biomass. It is a secondary technique to quantify the bacterial density and is also considered valuable for evaluating the performance of CWMs (Truu et al., 2009). Consequently, the MBC may provide valuable information about the structure and community of microbes in CWMs. Various researchers have mainly focused on the relevance of MBC towards the ecological parameters, such as seasons, land use and macrophytic species (Calheiros et al., 2009a). Several earlier works have evaluated that the microbial populations exhibited various features for the removal of wastewater contaminants (Krasnits et al., 2009). Such microbial populations have been observed as key components for the water quality improvement (Faulwetter et al., 2009). Numerous other aspects strongly influence the microbial community structure such as temperature, macrophytic diversity, hydrologic conditions and biotic succession (Zhang et al., 2011). The popular bacterial inhabitants are present in the rhizosphere area. It is described that the *Nitrosomonas* bacteria is the main inhabitants in the root zone of the macrophytes, responsible for the acceleration of nitrogen removal (Puigagut et al., 2008). Several macrophytic specieses promote the growth of some specific bacterial populations. For that, halotolerant macrophytes are capable of enhancing the performance via liberating O_2 and promoting the growth of halotolerant bacteria (Wu et al., 2009; Xiong et al., 2011; Wu et al., 2012). The presence of microbial communities in soil substrate within CWMs treating DW was identified previously (Truu et al., 2005). The depth of wetland is the main factor that influences the action and structure of bacterial communities (Truu et al., 2009). Numerous other studies have described the range of microorganisms in full and laboratory scale CWMs (Dong and Reddy, 2010; Zhang et al., 2010). Though, the data about the shift in the microorganism's diversity for long-standing wetlands is still insufficient (Adrados et al., 2014). The roots of macrophytes assist in the development of microbial community structures (Faulwetter et al., 2013). It is also reported that the root exudates from various macrophytes like *Thalia dealbata* influence the development of Cyanobacteria (Zhang et al., 2011). Occurrence of extra nutrients and other toxic materials in the wastewater negatively impacts the development of biofilms and their structure (Giaramida et al., 2013). A total of 180 aerobic and anaerobic bacteria were isolated from the rhizosphere region of planted and unplanted CWs by Llanos -Lizcano et al. (2019). A study carried out previously also exhibited parallel results about the microbial populations within natural and CWMs (Peralta et al., 2013). They observed that the *α-Proteobacteria* was the leading class, along with *γ-Proteobacteria* and *β-Proteobacteria*. According to the 16S rRNA gene sequencing, the microbial populations in CWMs having dissimilar loads of phosphorus are characterized using LH-PCR by Ahn et al. (2007). This study shows that the *α-Proteobacteria* (about 48-60%) are dominant in sediment followed by *Actinobacteria* and *Firmicutes*. Various other investigators have also assessed several associations amongst microbial diversity and removal efficacies in CWMs (Wu et al., 2016; Zhang et al., 2016).

2.5 Microbially mediated reactions within CWMs

The most prominent mechanism of pollutants removal operational in CWMs is filtration. Together with filtration, several other microorganism-assisted mechanisms such as photo-degradation, transpiration flux, sorption, sedimentation, volatilization and macrophytic uptake also applied together to enhance the removal performance (Morvannou et al., 2014). Several bacteria in animal waste actively participate for the breakdown of organics and also reduce the pathogens. Chemolithotrophic bacteria are directly involved in the biological degradation of ammonium (NH_4^+-N). They are accountable for the alteration of NH_4^+-N into nitrate (NO_3^--N) via nitrogen cycling (Ansola et al., 2014). The impact of urban wastewater on the functioning and structure of communities of these bacteria has been studied using DGGE by Oved et al. (2001). They observed a considerable change in the community's structure of these bacteria with *Nitrosomonas* as a dominant *spp.* in CWMs as compared to others. Consequently, it may be stated that diversity of ammonia-oxidizing bacteria has an important role (Rowan et al., 2003). Principally, there are six main biological methods working in the removal process, these are nitrification, denitrification, microbial phosphorus exclusion, photosynthesis, respiration and fermentation (Mitchell and McNevin. 2001). Effluent that is severely laden with BOD and COD generally involves O_2 and sulphur loving bacteria in the process of oxidation and methanation (Chan et al., 2020). O_2 pass in the substrate bed with the help of roots of macrophytes and develop aerobic zone. This section has abundance of *Nitrosomonas* and *Pseudomonas aeruginosa spp.* that are responsible for the aerobic breakdown of pollutants. However, sulphur degrading bacteria and methanogens are dominant in the anaerobic zone. Anaerobic degradation of pollutants mainly occurs via fermentation and methanogenesis (Pedescoll et al., 2011). The performance of CWMs is mainly regulated by the available microbial inhabitants and their metabolic action (Wetzel, 1993). The action of microbial populations results into conversion of numerous harmful materials into harmless unsolvable constituents and modifies the redox responses of the substrate materials. Aerobic settings are more effective towards the elimination of majority of organic contaminants. It is observed that the nitrification-denitrification rate within CWMs with more than one macrophyte are much advanced as compared to one or no macrophyte (Hua et al., 2017). The distribution, type and density of the macrophytes can disturb the pollutants elimination mainly via the changing bacterial groups and successive aerobic and anaerobic regions (Zhu et al., 2017).

2.6 Shift in microbial communities depending upon seasons and macrophytes

The rhizosphere area is well recognized for rich microbial actions such as macrophyte– microorganism interactions and interface with substrate materials and contaminants. It is the zone where most of the biotic reactions happening because of close association among substrate and plant roots. It is also known as a region with diverse elements such as organic acids, vitamins, minerals, carbon compounds, sugars, phenol, polysaccharides and enzymes and numerous other constituents that promote microbial degradation of organic contaminants (Bertin et al. 2003; Faulwetter et al. 2009). The existence of macrophytes provide the growth medium for the development of rich microbial colonies around the rhizosphere regions (Wang et al., 2016). During the winters, the arrangement of microorganisms is wide due to the irregular bacterial affluence. However, in summer months, CWMs planted with *Phragmites australis* have more *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* and unplanted units have mainly *Cyanobacteria* and photosynthetic bacteria (Wang et al., 2016). It is reported that *Thalia geniculata* can increase the DO via well-organized aerenchyma within the roots (Longstreth and Borkhsenious, 2000; Yovo, 2016). Impact of both microorganisms and macrophytes on the exclusion of nutrients within CWMs as described earlier has been provided in table 2.3. Several macrophytes such as *Cyperus* also excrete bactericidal exudations within the rhizosphere (Alufasi et al., 2017), *Cyperus articulatus* are capable of offering excess O_2 to the rhizosphere and provide an effective exclusion of nitrogen (>75%). However, the consequences with the same species may vary depending upon season and working settings (Shelef et al., 2013). From these studies, it is clear that in CWMs, microbial populations are vital for biogeochemical responses to improve the water quality (Saunders et al., 2013). A slight adjustment in the microorganism’s community structure might alter the removal capacity. In winter season, the microbial activity generates adequate heat to protect the biofilms from freezing. The rate of breakdown of contaminants become slow with decrease in temperatures therefore; the decomposition rates may be enhanced by increasing the dimensions of wetland system. It is also reported that in summer season, the diversity of bacterial populations has negative correlation with the removal of organics and nutrients mainly at high population size and diversity (Tian et al., 2017). Some anaerobic facultative bacterial inhabitants are able to work efficiently under the both aerobic and anaerobic circumstances with changing environmental settings. However, several bacterial inhabitants during unfavorable environments, become dormant and remain inactive for several years (Hilton, 1993). It is observed that specific population of microorganisms are accountable for the breakdown of particular pollutants (Faulwetter et al., 2009). Consequently, the knowledge about the association of microbial communities is beneficial to reveal the pollutant’s elimination pathways in CWMs.

Table 2.3 Impact of microorganisms and macrophytes on the exclusion of nutrients within CWMs

Country	Wastewater	Macrophytes	Microorganisms reported	Removal efficiency (%)		Reference
				NH_4^+-N	PO_4^{2}-P	
USA	Synthetic	*Schoenoplectus tabernaemontani*	*Actinobacteria, α-Proteobacteria* (around 48–60%) and *Firmicutes*	NA	79	Ahn et al. (2007)
	Run-off	NA	α-Proteobacteria was the dominant followed by γ- and β-Proteobacteria	NA	NA	Peralta et al. (2013)
	Synthetic		*Actinobacteria, Acidobacteria, Firmicutes, Armatimonadetes, Nitrospirae, Bacteroidetes, Chlorobi, Chloroflexi, Parcubacteria Gemmatimonadetes, Proteobacteria, Ignavibacteriae, Hydrogenedentes, Planctomycetes,* and *Verrucomicrobia*	NA	NA	Li et al. (2019)
China	Sewage	*Kandelia candel*	*Nitrosomonas, Vibrio, Candidatus Planctomyces, Competibacter, Marinobacterium, Denitratisoma, Nitrospira, Thauera, Dechloromonas* and *Magnetospira*	74.9	NA	Fu et al. (2020)
	Swine	*Myriophyllum aquaticum*	*Proteobacteria, Firmicutes, Bacteroidetes, Chloroflexi, Actinobacteria, Cyanobacteria, Acidobacteria, Planctomycetes, Deffeibacteres and Verrucomicrobia*	92.34	NA	Xu et al. (2020)
Colombia	Domestic	*Cyperus articulatus* and *Thalia geniculata*	Total 180, out of which 65 was anaerobic and 115 was aerobic heterotrophic bacteria	83 83	74 74	Llanos-Lizcano et al. (2019)
Denmark	Domestic	*NA*	*Flavobacterium* sp., γ-Proteobacteria, *Arthrobacter* sp., *Thauera terpenica, Xanthomonas* sp., *Stenotrophomonas* sp., *Dokdonella* sp., and *Rhodanobacter* sp.	60	NA	Adrados et al. (2014)

*NA = Not Available

2.7 Interspecific competition among macrophytes

Interspecific competition among macrophytes to access light, nutrients and oxygen for their growth is vital to determine wetland vegetation (Gioria and Osborne, 2014; Zheng et al., 2016). The assemblage of different macrophytes in mixed culture induces stress on adjoining macrophytes (Craine and Dubinsky, 2013). The uptake of nutrients and assimilation of several other wastewater contaminants by macrophytes planted in mixed culture within CWMs is a key area of concern (Zheng et al., 2016). Dissimilar characters of macrophytes enable the competition for resources depending upon nutrient availability (Martin and Coetzee, 2014). In wastewater containing excess-nutrient, the growth of macrophytes becomes higher and competition for incoming light gets more crucial (Gioria and Osborne, 2014). However, in poor nutrient condition, the growth of macrophytes become slow and competition may shift to belowground for the enhancement in the root growth to acquire more nutrients (Gioria and Osborne, 2014). Several ecological settings and nutrient accessibility are key determining factors of competitive capability of a particular species (Mony et al., 2007). Additionally, several morphological features of macrophytes such as large canopy diameter, leaf shape and tall shoot are act as overriding factors for improved competitive capacity (Kankanamge and Kodithuwakku, 2017). It is reported that due to the high inconsistency in interspecific competition of planted macrophytes, the growth and steadiness of various macrophytes in mixed culture for continuing operation stay uncertain (Agami and Reddy, 1990; Amon et al., 2007). Studies dealing with competitive interaction among macrophytes planted in the mixed culture within FWS and SSF CWMs under the identical ecological settings and influent load at the field scale are still not available. The *Phragmites spp.* and *Typha spp.* are the popular macrophytes utilized in CWMs due to their high reproduction and flood-tolerant capabilities (Vymazal, 2013a). Yet, the application of these two macrophytes in mixed culture within CWMs is rare.

2.8 Enzyme activity

The activity of extracellular enzymes inside the root zone of macrophytes utilized in the CWMs has been considered very crucial to obtain the information about the role of microbial inhabitants (Zhang et al., 2011). The structure of bacterial community has been associated to with enzyme actions (Zhang et al., 2007). Activity of several enzymes in soil substrate within CWMs is also recognized as a vital parameter to enhance the water quality (Kang et al., 1998; Martens et al., 1992; Shackle et al., 2000). Several biological and edaphic factors such as microbial communities, fauna, pH, texture, higher taxa, depth, organic matter and nutrient composition influence the activity of numerous enzymes inside the substrate materials (Reboreda and Cacador, 2008; Kumar and Dutta, 2019b). The enzymatic activity may also be changed by altering the supply of carbon to improve the performance of CWMs as described by Shackle et al. (2000). They concluded that addition of extracellular enzymes may also advance the biodegradation process of contaminants. The activity of several enzymes such as dehydrogenase (DHA), urease, phosphatase, cellulase, fluorescent diacetate (FDA), microbial biomass carbon (MBC) and protease showed great variations depending upon the time, type of macrophytes and substrate depth (Kong et al., 2009; Kumar et al., 2020a). The action of urease in CWMs planted with *Phragmites australis* has been always found higher as compared to several other macrophytes. It is also reported that the activity of different enzymes declines with rise in substrate depth and remain higher in the uppermost layer (Niemi et al., 2005; Kumar et al., 2020a). Root's activity of macrophytes within rhizosphere zone possess a great correlation with the activity of enzymes (Kong et al., 2009). Macrophytes in the wetlands system are able to influence the activity of enzymes through changing the diversity and composition of microbial species via excreting enzymes, oxygen and exudates. These are also functioned for the reactivation of enzymes through oxygenation which may be deactivated and stored by tannin with various other elements in anoxic zone (Neori et al., 2000).

2.9 Reuse of treated wastewater

The reuse of treated DW for several activities such as irrigation, improving the base flow of rivers and for gardening etc. is a practical solution that can provide an alternative water resource especially in water deficient regions. After suitable treatment, it may be utilized to reduce the water stress by improving groundwater table via recharge. It can also be used for the production of agricultural crops and in maintaining the greenbelts to lessen the dependency on fresh water reservoirs (Declercq et al. 2020). Discharging treated water in freshwater bodies to sustain the aquatic life can provide a healthy aquatic ecosystem. Together with these, several ecological services such as fisheries, valuable wildlife, aesthetic values and recreational uses are also encouraged by treated wastewater (Kumar and Dutta 2019b).

2.10 Economic feasibility of CWMs

Almost all available wastewater treatment methods are dependent on sedimentation, filtration, biological action etc., and are usually developed with complex mechanical structures that requires high energy inputs (Stefanakis, 2020). Numerous other conventional methods such as UASB, ASP, membrane bioreactors and FAB etc. are also utilized efficiently for the DW treatment. However, the high-cost requirement for the operation and maintenance of these systems and the long-lasting field scale application of these techniques are not viable (Zhang et al., 2021). CWMs are recognized as alternative biological treatment methods to traditional wastewater treatment systems principally for decentralized and remote locations (Kumar and Dutta, 2019b). The excellent pollutant removal efficiency of CWMs together with eco-friendly and sustainable design are some of the favorable parameters that play a major role in their wider application. They require low operational and working costs as compared to facultative or oxidation ponds (Stefanakis, 2020). CWMs depend fully on natural environmental forces under specific input conditions and need less energy. The appropriate removal efficacy together with several environmental paybacks such as flood control, groundwater recharge, fisheries, wildlife habitats, aquaculture, carbon sequestration, recreational uses and silt detention make them attractive and cost-effective wastewater treatment technology (He et al., 2018).

Chapter 3

Performance of CWMs with special reference to wastewater nutrients and heavy metals removal

3.1 Introduction

Lack of suitable wastewater treatment technologies, rapid urbanization and population growth contribute to discharge of the majority of untreated or partially treated wastewater into freshwater streams (Kumar and Dutta, 2019b). Wastewater originates from various small industries and household activities especially in developing countries is laden with several HMs and other contaminants. Such wastewater significantly damages the aquatic life of receiving waterbody and human beings through the buildup in the sediments and through percolating in the groundwater (Ali et al., 2013). Various technologies have been applied for the remediation of wastewater such as membrane filtration, nanotechnology, bioreactors etc. with ecological friendly materials for the exclusion of HMs and other contaminants (Bavandpour et al., 2018). However, the use of these technologies is not feasible on field scale particularly due to their high working and maintenance cost (Wu et al., 2020; Zhang et al., 2020). Several physicochemical and biological methods have been also applied in the removal of wastewater pollutants, together with HMs (Khalifa et al., 2020; Zheng et al., 2020). In CWMs, physical and biological processes are primarily responsible for the elimination of wastewater nutrients. Filtration and sedimentation are most common physical methods utilized in the treatment with the support of planted macrophytes and substrate materials (Sundaravadivel and Vigneswaran, 2001). However, the key biological methods comprise of photosynthesis, respiration, nitrification, denitrification and fermentation. Photosynthesis tends to sustain the oxygen supply, however; respiration sustains the concentration of DO. Nitrification, denitrification and fermentation function to eliminate nitrogen (N_2) and organics respectively (Kumar and Dutta, 2019b). Removal of phosphorus mainly proceeds by adsorption with the assistance of iron, calcium and magnesium ions present in the substrate material.

In developing nations such as India, it is a common tendency to mix industrial waste with domestic and agricultural runoff (Kumar et al., 2020c). Such mixing of DW and industrial effluent containing HMs enhances the complexity and cost of treatment (Yadav et al., 2012). To avoid the mixing of HMs into freshwater ecosystems, there is a growing concern for the treatment of mixed wastewater in urban as well as in rural localities (Ding et al., 2018; Zhang et al., 2020). However, earlier findings revealed that CWMs have numerous drawbacks when operated for HMs removal. The removal efficiency differs significantly ranging from 20% to more than 90%, based on the design of CWMs, type of macrophytes, substrate and metal species (Gill et al., 2014; Nuamah et al., 2020).

From these findings, it is estimated that the toxicity of metals on microbial development and macrophytes growth might influence the removal capacity and biodegradation of organics and nutrients (Kumar and Dutta, 2019b). The performance of CWMs towards metals removal is studied through retention time, precipitation and co-precipitation, macrophyte uptake and microorganism's metabolism (Marchand et al., 2010; Vymazal and Brezinova, 2016). The translocation factor (TF) and bioconcentration factor (BCF) are two vital parameters that are used to assess the capability of macrophytes for metal phytoremediation (Katukiza et al., 2014). BCF signifies the metal uptake ability of plants, whereas TF characterizes the internal transportation of metals from roots to both leaves and stems (Zhang et al., 2020). In this chapter, I have discussed the overall removal performance of CWMs particularly for the removal of BOD, TP, SRP, NH_4^+-N, NO_3^--N and NO_2^--N and HMs such Pb, Cd, Cr, Zn, As, Cu, Ni and Iron Fe. I have also evaluated the impacts of dissimilar macrophytic combinations and HRTs on the removal capability of selected water quality parameters including HMs. The BCF, TF, RCF and ATCF for all selected HMs of planted macrophytes were also calculated.

3.2 Material and Methods

3.2.1 Description of the CWM units

The whole research work was conducted in the green house of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow (26.7697° N, 80.9262° E) UP, India. Eight CWM units were designed in concrete containers (dimension 1.2 x 0.60 x 0.76 m length, width and depth respectively). Each CWM unit had 8 * 8 * 16 cm layer of crushed stone, sand and soil respectively as a filter material. However, total three emergent and free-floating macrophytes were utilized in this study, these were *Phragmites karka, Typha latifolia* and *Pistia stratiotes*. *Pistia stratiotes* and *Typha latifolia* were collected from the university campus and *Phragmites karka* from the adjacent areas of Lucknow District. Macrophytes were selected according to their contaminants uptake capability as evaluated in several previous studies (Rezania et al., 2019; Kumar et al., 2020a; Kumar et al., 2021; Zhang et al., 2021).

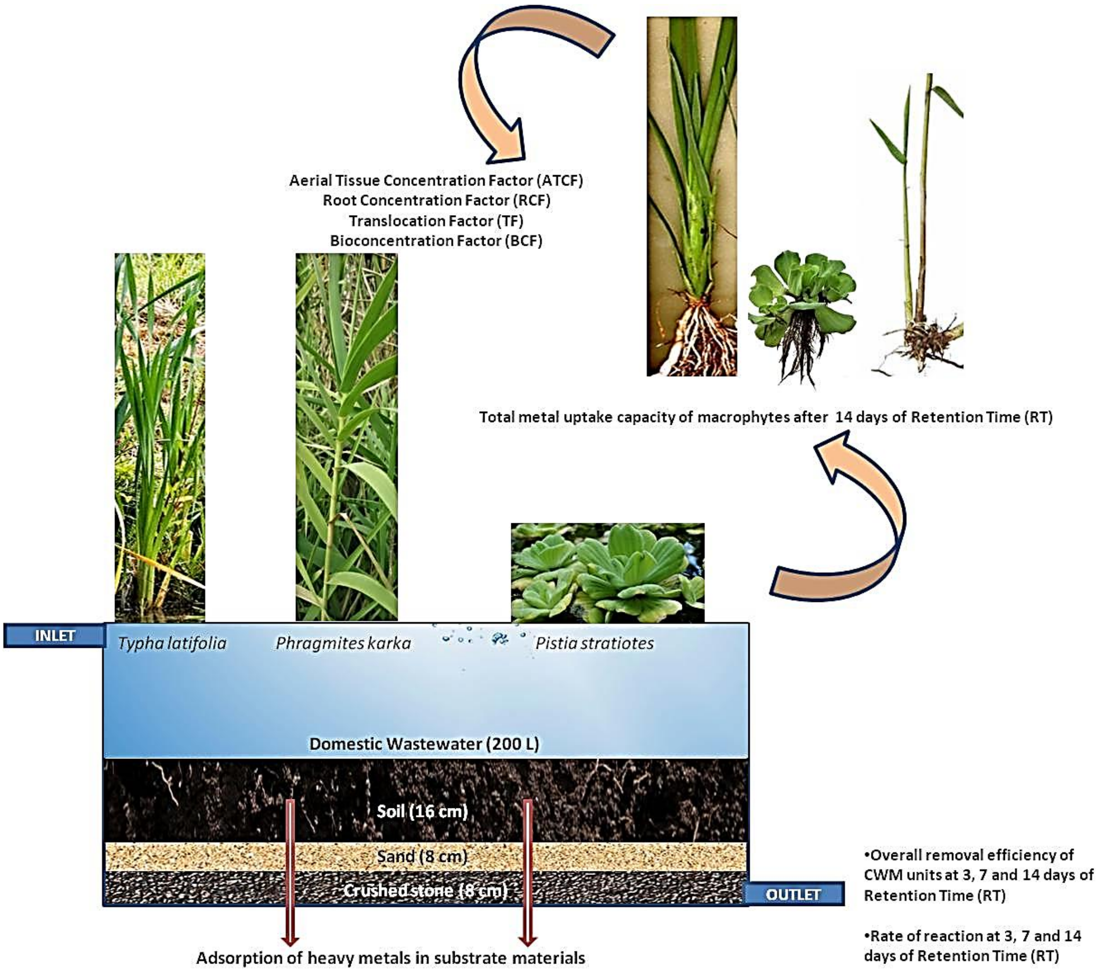


Fig. 3.1 Illustration of the mixed assembly of macrophytes in the CWM unit

Macrophytes that had almost similar weight and height were planted as single as well as in combinations. On the basis of macrophytic plantation, the eight CWM units were Pi, Ph, T, Ph+T, Pi+T, Pi+Ph, Pi+Ph+T and an unplanted unit as control. The pictures of initial phase of construction and working of CWM units have been given in fig. 3.2 and 3.3 respectively. The initial density of macrophytes was 18 plants per CWM unit. All the units were filled with tap water after the plantation of macrophytes and left for 30 days for the acclimatization and stabilization. After that, the raw DW was collected from a wastewater drain in a plastic container having 200 liters capacity, transported at site and filled in all CWM units equally. Each CWM unit held 200 L of DW as batch feeding. The experiment was conducted from March, 2018 to February, 2020 for the analysis of selected water quality parameters, however, for the analysis of HMs, the experiment was done for one year during the October, 2018 to September, 2019. The physico-chemical characteristics of DW have been given in table 3.1. Several environmental factors such as relative humidity (RH), temperature and solar intensity were measured on the daily basis. Humidity and temperature were measured by Huger Thermo-hygrometer (8270) and solar intensity through the Luxmeter (LX-101A) provided by HTC^{TM}. Analysis of DO and pH was also performed to know the daily variation between different CWM units via Lutron (DO-5509) and Hanna (Hi96107) portable meter respectively. The electrical conductivity (EC) and TDS of wastewater was measured by HM digital meter (TDS-3).

3.2.2 Effluent samples collection and their analysis

The effluent samples were collected in glass bottles (500 mL) in triplet from each CWM units after 3rd, 7th and 14th day respectively. All the selected parameters of effluent samples were measured according to the standard methods prescribed by the APHA and CPCB guide manual for water and wastewater analysis (APHA, 2017). BOD was determined by 5-day BOD test and NO_3^--N, NO_2^--N and NH_4^+-N were measured by ultraviolet (UV) spectrophotometric colorimetric method. However, TP and SRP were determined using stannous chloride colorimetric method. All UV- visible spectrophotometric analysis was performed using UV- Visible Systronics - 2203 spectrophotometer.

Table 3.1 Characteristics of raw domestic wastewater (DW) (mean ± SD, n=24)

DW	**Biochemical Oxygen Demand (BOD)**	**Total Phosphorus (TP)**	**Soluble Reactive Phosphorus (SRP)**	**Ammonium (NH_4^+-N)**	**Nitrate (NO_3^--N)**	**Nitrite (NO_2^--N)**	**pH**	**Electrical conductance (EC*)**	**TDS**
Concentration (mg/L)	108.11± 11.69	11.67 ±2.85	8.07 ± 2.60	25.56 ±6.26	12.38 ± 4.56	4.88 ± 2.08	5.49 ± 1.52	0.96 ± 0.15	480 ± 52

*EC (mS/cm^2)

Analysis of all selected HMs such as Pb, Cd, Cr, Zn, As, Cu, Ni and Fe was done by digestion (except for As) on a hot plate using nitric acid and perchloric acid (5:1 ratio) (Mant et al., 2006). After that, the digested samples were filtered out using Whatman

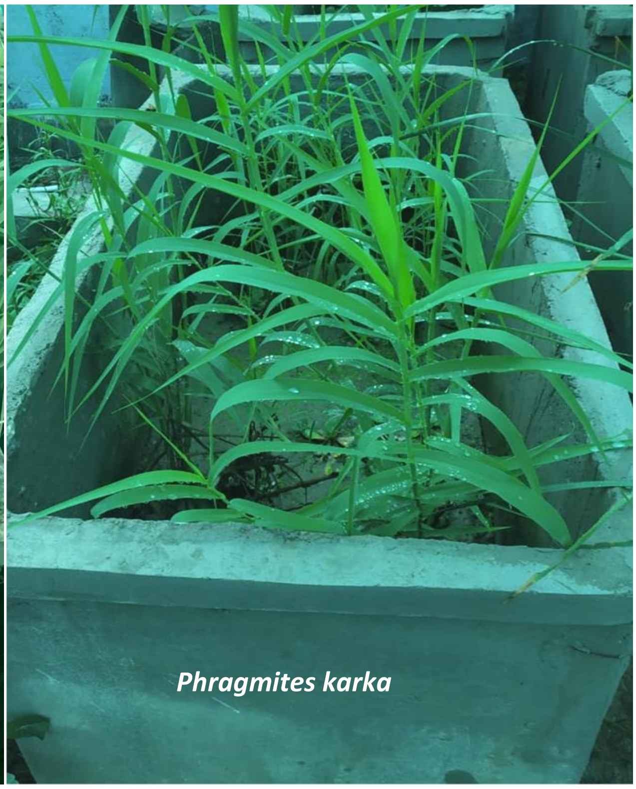
(0.45µ) filter and analyzed through ICP-MS (iCAP TQ). The initial concentrations of all selected HMs have been given in table 3.2.

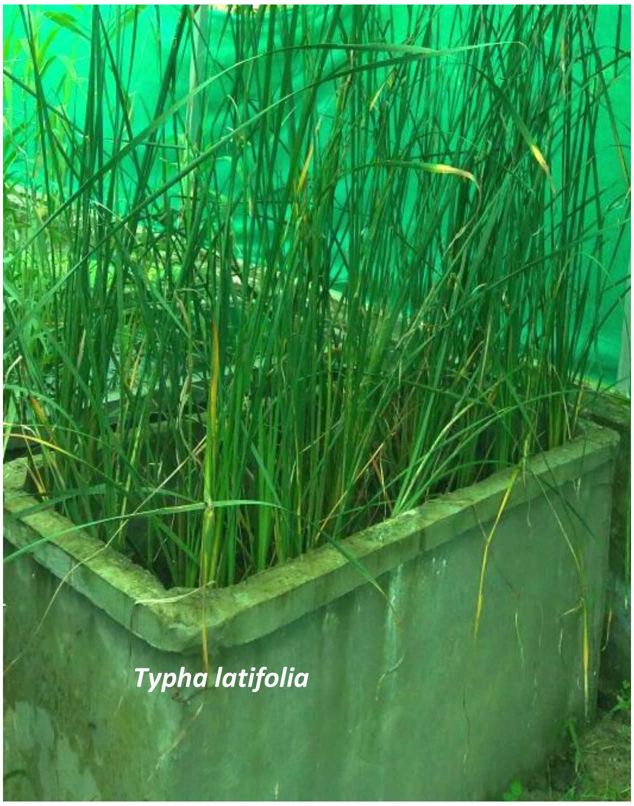
Table 3.2 HMs concentration in raw DW (mean ± SD, n=12)

S. no.	HMs	Concentration (mg/L)
1	Chromium (Cr)	1.2 ± 0.001
2	Cadmium (Cd)	1.1± 0.002
3	Lead (Pb)	5.23 ± 0.056
4	Zinc (Zn)	17.85 ± 0.012
5	Copper (Cu)	2.3 ± 0.004
6	Manganese (Mn)	3.96 ± 0.048
7	Arsenic (As)	1.44 ± 0.002
8	Iron (Fe)	20.19 ± 2.85
9	Nickel (Ni)	3.60 ± 0.003

Fig. 3.2 Macrophytes sapling and CWM units during the initial phase of design that are filled with tap water for the stabilization of substrate layers and macrophytes


Pistia stratiotes


Phragmites karka


Typha latifolia


Phragmites karka + Typha latifolia

Fig. 3.3 Eight working CWM units in the green house of Department of Environmental Science, BBAU, Lucknow for DW treatment

3.2.3 Macrophytes sample collection and their analysis

Macrophyte samples were collected after 14-day retention time, separated into root and aerial parts, washed to remove debris and examined for the HMs concentrations as explained by Mant et al. (2006). An experimental blank of soil substrate from control unit was also performed to lessen the error for background HMs availability. Bioaccumulation of HMs in roots and aerial parts of macrophytes was studied to understand the uptake capacity of particular macrophyte. For that, the BCF, TF, ATCF and RCF for all selected HMs within all three macrophytes were measured.

3.2.4 Calculation

The removal efficiency (RE) of all selected parameters including HMs was calculated as-

RE (%) = (1 – Ce/Ci) *100 (9)

Where, C_e and C_i stand for effluent and influent concentrations respectively

Bioconcentration factor (BCF)= $(C_A+C_R)/C_S$ (10)

Translocation factor (TF) = C_A/ C_R (11)

Where, C_A, C_R and C_S signifies the concentrations of HMs in aerial parts, roots of macrophytes and in soil substrate respectively (Stoltz and Greger, 2002; Deng et al., 2004; Soda et al., 2012).

Aerial tissue concentration factor (ATCF) = C_A/C_I (12)

Root concentration factor (RCF) = C_R/C_I (13)

Here, C_I, C_A and C_R signifies the concentration of HMs in wastewater, aerial parts and roots of macrophytes.

Second-order rate constant (k) = 1 $(A_0-A)/(t*A*A_0)$ (14)

Here, A_0 and A signifies the initial and final concentrations of HMs at t time.

3.2.5 Statistical analysis

The analysis of the data was completed via MS office Excel and SPSS (version 2016 and 20 respectively). All the data utilized in this work are presented as the mean ± SD. Analysis of variance amongst the mean removal of HMs, their decay constant and several other selected parameters with respect to time and various CWM units were done by one-way ANOVA ($p < 0.05$). The second-order kinetics and Pearson correlation coefficient were also used to measure the decay rate of HMs depending upon time and to assess the effects of each other parameters on removal efficacy respectively.

3.3 Results and Discussion

3.3.1 Environmental conditions

The temperature ranged from 27 to 36.3^{0}C, solar intensity from 101*100 to 512*100 Lux and relative humidity from 66 to 90% respectively annually. The temperature, relative humidity and solar intensity were evaluated to know daily variation among these and their impacts on the removal efficacy of CWMs (Table 3.3). The growth rate of planted macrophytes improved greatly during the summer as temperature and relative humidity were higher (Licata et al., 2019). The average DO and pH of the DW which is used throughout the experiment were 1.20 ± 0.52 mgL^{-1} and 5.48 ± 0.52 respectively. In this study, it is observed that the concentration of DO differ widely between several CWM units due to the dissimilar macrophytic combinations that support the growth and development of diverse microbial groups (Zhang et al., 2010; Zhang et al., 2020). It is well established that microbial inhabitants existing in CWMs consume DO for the transformation of organic materials. Primarily, the DO declined rapidly due to the chemical oxidation and aerobic respiration (Ding et al., 2018; Xu et al., 2020). Thereafter, an increasing trend was recorded in almost all CWM units as majority of organics were transformed and consumed by microbial populations as discussed in previous study (Ding et al., 2011). The CWM unit having *Pistia stratiotes* and *Phragmites karka* (Pi+Ph) attained maximum DO as compared to others throughout the experimental period (Fig 3.4). The value of pH also improved from 5.48 to 7.5 after 14-day HRT. It is reported in several previous studies that the higher temperature supports the growth and activity of microbial populations that play crucial role in the breakdown of pollutants (Kadlec and Reddy, 2001; Kadlec and Wallace, 2009; Kumar and Dutta, 2019a).

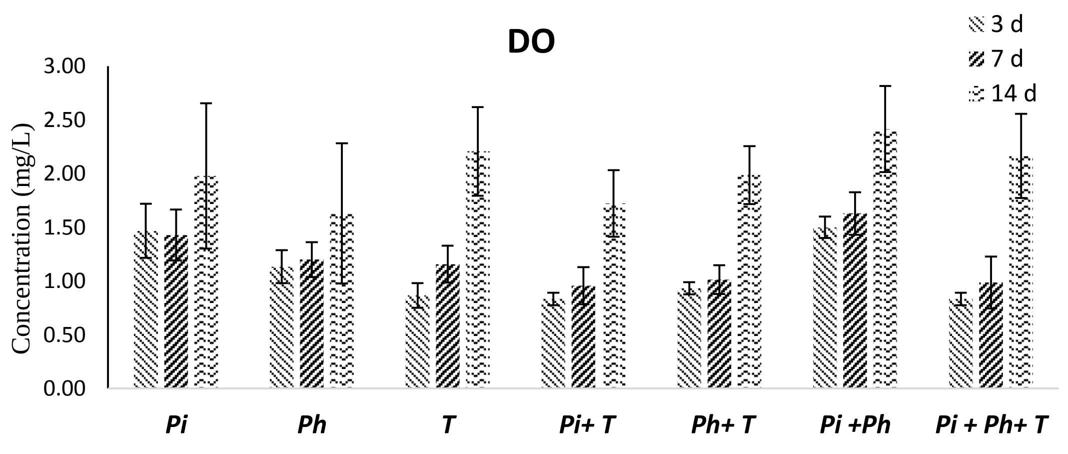


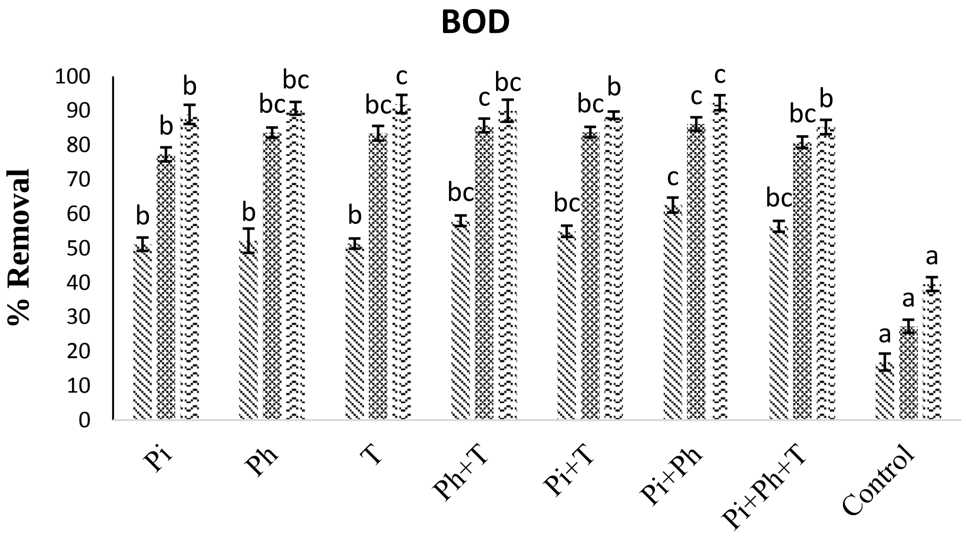
Fig. 3.4 Average DO among various CWM units at 3, 7- and 14-days HRT throughout the study (mean ± SD, n= 24)

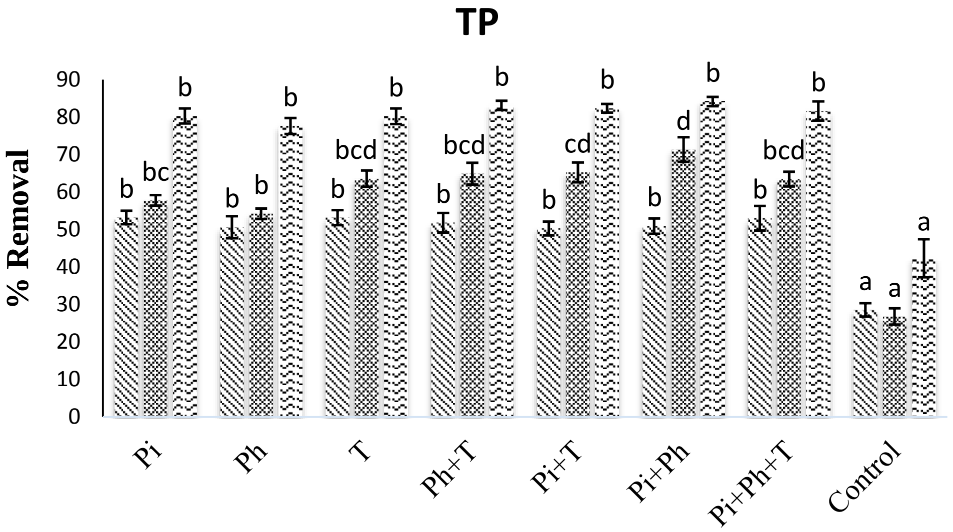
Table 3.3 Dailly average relative humidity, solar intensity and temperature throughout the experiment (mean ± SD, n = 24)

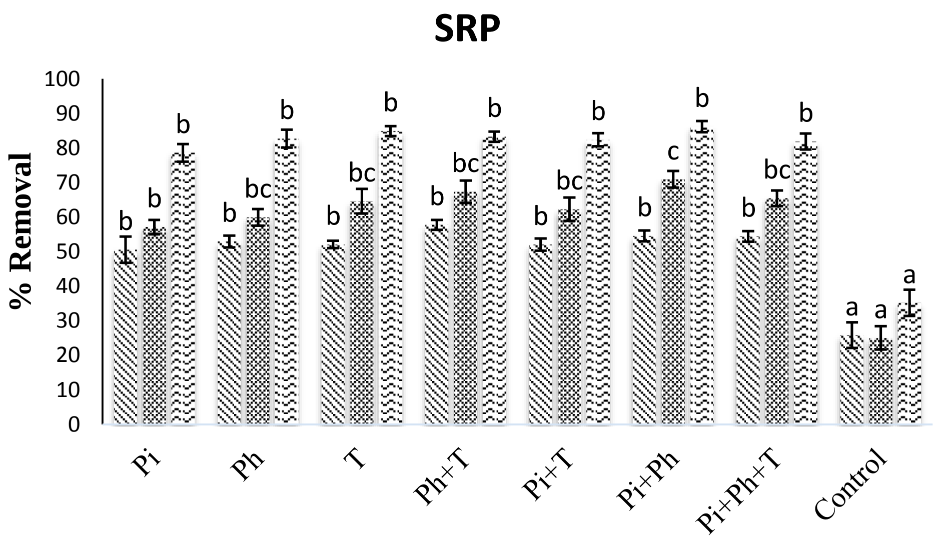
Days	Relative humidity (%)	Average Solar intensity (Lux)	Average Temperature (^{0}C)
1	66 ± 10	45000 ±1700	34 ± 4
2	77 ± 08	44400 ± 1600	32 ±3
3	90 ± 07	25000 ± 800	28 ± 5
4	93 ± 12	33700 ± 1000	27 ± 7
5	83 ± 09	48800 ± 1500	30.3 ± 2
6	72 ± 05	37600 ± 1300	31.2 ± 2
7	73 ± 06	51200 ± 2000	33 ± 3
8	71 ± 08	35700 ± 1400	34 ± 3.5
9	75 ± 09	136100 ± 2100	29 ± 4
10	85 ± 12	21900 ± 1300	32 ± 5
11	84 ± 10	12100 ± 2400	30.5 ± 4
12	76 ± 08	25700 ± 1600	29 ± 6
13	80 ± 08	25400 ± 1200	31 ± 3
14	82 ± 09	26800 ± 1100	32 ± 2
15	79 ± 07	42100 ± 1800	34 ± 2
16	78 ± 11	29500 ± 900	31 ± 3
17	77 ± 10	34900 ± 1000	32 ± 3.5
18	71 ± 15	37700 ± 800	33 ±2
19	72 ± 14	34800 ± 1100	32 ± 2
20	85 ± 12	38200 ± 1300	33 ± 1
21	87 ± 14	34500 ±1200	32.2 ± 2
22	86 ± 16	47500 ± 1600	34 ± 4
23	88 ± 13	45400 ± 1400	36.3 ± 5
24	89 ± 20	36200 ± 1200	31.8 ± 5
25	90 ± 22	12000 ± 2300	31 ± 4
26	82 ± 12	10100 ± 2500	30 ± 4
27	84 ± 07	20400 ± 1300	33 ± 3
28	83 ± 08	39000 ± 600	35 ± 4
29	81 ± 11	32100 ± 500	33 ± 3
30	85 ± 14	39500 ± 1400	34 ± 2

3.3.2 Removal efficacy of wastewater contaminants depending upon CWMs

The average removal efficacy for TP, SRP, BOD, NH_4^+-N, NO_2^--N and NO_3^--N amongst dissimilar CWM units planted in single as well as in mixed culture of macrophytes with respect to different HRTs are exhibited in fig 3.5. Variance analysis ($p < 0.05$) among the mean removal capacity for different pollutants in several CWM units displayed significant difference among removal efficacies depending upon HRTs and CWM units. The highest removal of BOD was revealed by CWM unit Pi+Ph (62.58, 86.14 and 92.34% for all three HRTs respectively). Removal of TP extended from 50 to 53%, 54.19 to 71.32% and 77.62 to 84.14% between different CWM units for 3-, 7- and 14-day HRTs respectively. However, the maximum elimination ability was displayed by CWM units Pi (53.20%) for 3-day HRT and Pi+Ph (71.32 and 84.14%) for 7 and 14 day respectively. The other CWM units also exhibited good results as defined in earlier investigations (Zhai et al., 2016; Zheng et al., 2016; Kankanamge and Kodithuwakku, 2017; Hickey et al., 2018). It is observed that *Pistia stratiotes* and *Phragmites karka* are capable of removing phosphorus efficiently (Yasar et al., 2018). They also observed that the *Pistia stratiotes* possess higher removal efficacy towards several other physical as well as chemical pollutants. Correspondingly, higher exclusion efficacy for SRP was observed in CWM unit Ph +T (57.73%) for 3 d HRT and Pi+Ph (70.94 and 86.15%) for 7 and 14 day respectively. Removal of NH_4^+-N was also observed maximum in the same CWM unit (65.54, 75.89 and 87.02%) for all HRTs respectively. Parallel outcomes were also recorded in numerous earlier studies for the treatment of municipal wastewater (Hickey et al., 2018; Zhai et al., 2016). Again, the maximum removal ability of NO_3^--N and NO_2^--N was shown by Pi+Ph at all three HRTs as compared to other CWM units. The other treatment units also exhibited optimum elimination efficacies for all parameters taken into consideration throughout the experiment. The higher removal of wastewater nutrients may be the characteristic of these macrophytes to uptake more in their tissues as exhibited by *Phragmites karka* and *Pistia stratiotes* (Tanner, 1996; Jampeetong et al., 2012; Kumar et al., 2021).


BOD
% Removal
0
10
20
30
40
50
60
70
80
90
100
Pi
Ph
T
Ph+T
Pi+T
Pi+Ph
Pi+Ph+T
Control


TP
% Removal
0
10
20
30
40
50
60
70
80
90
Pi
Ph
T
Ph+T
Pi+T
Pi+Ph
Pi+Ph+T
Control


SRP
% Removal
0
10
20
30
40
50
60
70
80
90
100
Pi
Ph
T
Ph+T
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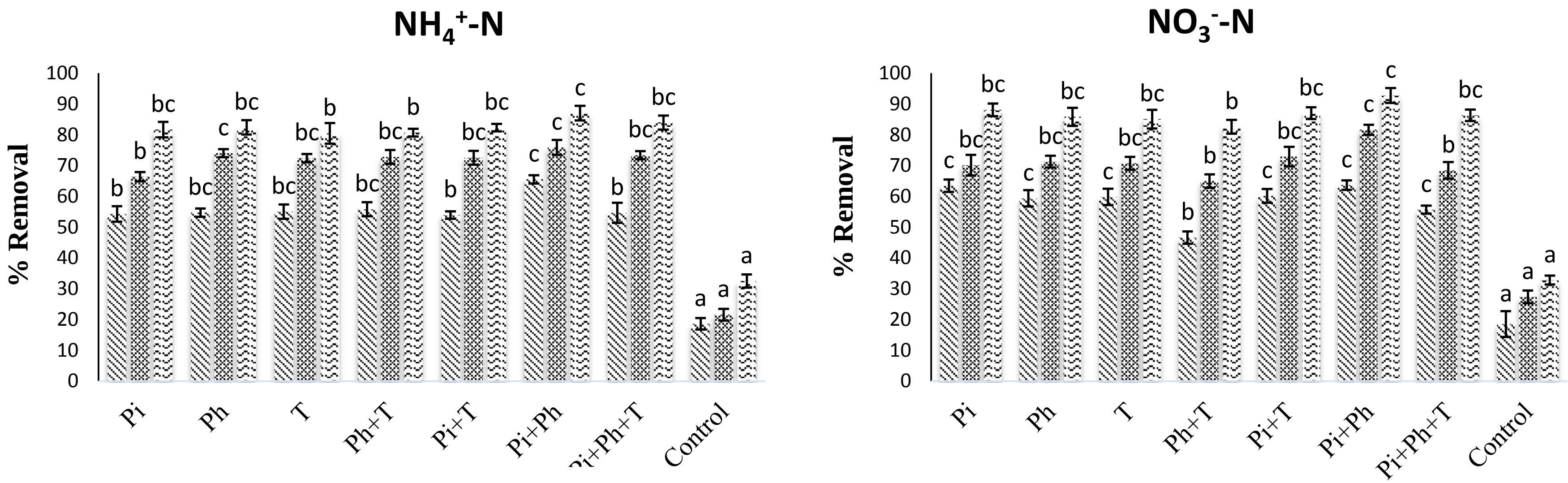
Pi+Ph
Pi+Ph+T
Control



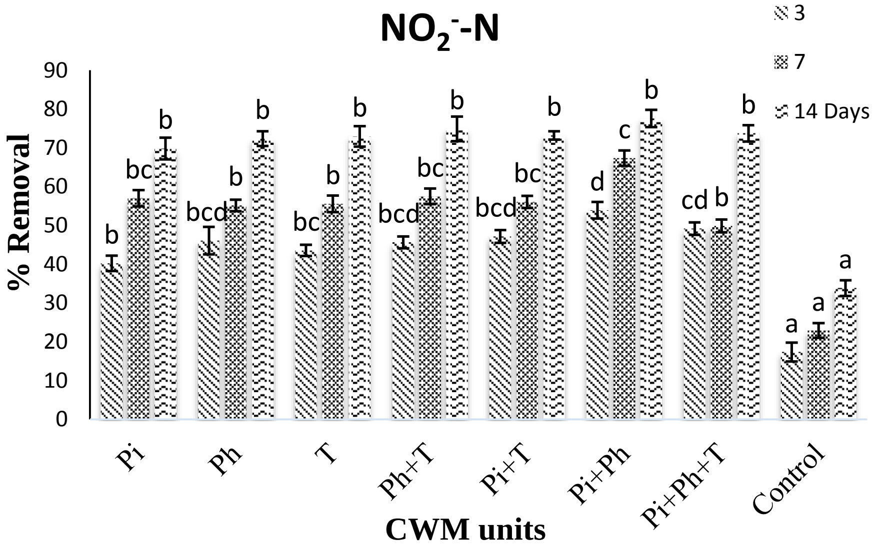


Fig. 3.5 Removal efficiencies for BOD, TP, SRP, NH_4^+-N, NO_3^--N and NO_2^--N of various CWM units at all three HRTs (mean ± SD, n= 24). Letters (a, b, c and d) as superscript signify the variance amongst the mean elimination efficacies

CWMs with emergent and free-floating macrophytes are considered as excellent choice for the removal of nutrients as well as HMs from wastewater via uptake as compared to CWMs with free-floating or emergent plants only (Vymazal, 2007). In our study, CWM unit having *Pistia stratiotes* and *Phragmites karka* (Pi+Ph) revealed higher removal performance at most of the time for all selected water quality parameters as compared to several other single and mixed units. All single planting units (except *Pistia stratiotes*) possess lower removal performances for nutrients as compared to mixed plantings units. Visual comparison of effluent quality between different treatment units has been provided in fig. 3.6. It is reported and also observed in present study that *Phragmites* are more efficient towards the removal of nutrients as compared to *Typha* as it offers adequate O_2 for aerobic breakdown and are able to grow vigorously and regenerate (Zheng et al., 2016). The removal efficiency can be enhanced further by intermittent aeration (Liu et al., 2019). Measurement of nutrients concentration in macrophytic tissues as plants per gram dry weights has lower significance concerning nutrient elimination capability, because it varies importantly by the extent of biomass (Zheng et al., 2016). Increased biomass of macrophytes and activity of enzymes inside the substrates at the same time, improved nitrogen elimination (Han et al., 2017; Sun et al., 2019). Use of mixed macrophytes in CWMs advances the removal performance of the system for various wastewaters (Toscano et al., 2015). The mixed planting systems were also recognized for their lower susceptibility to seasonal fluctuations (Liang et al., 2011; Chang et al., 2014). Nevertheless, the use of these mixed planting systems is inadequate on field scale.

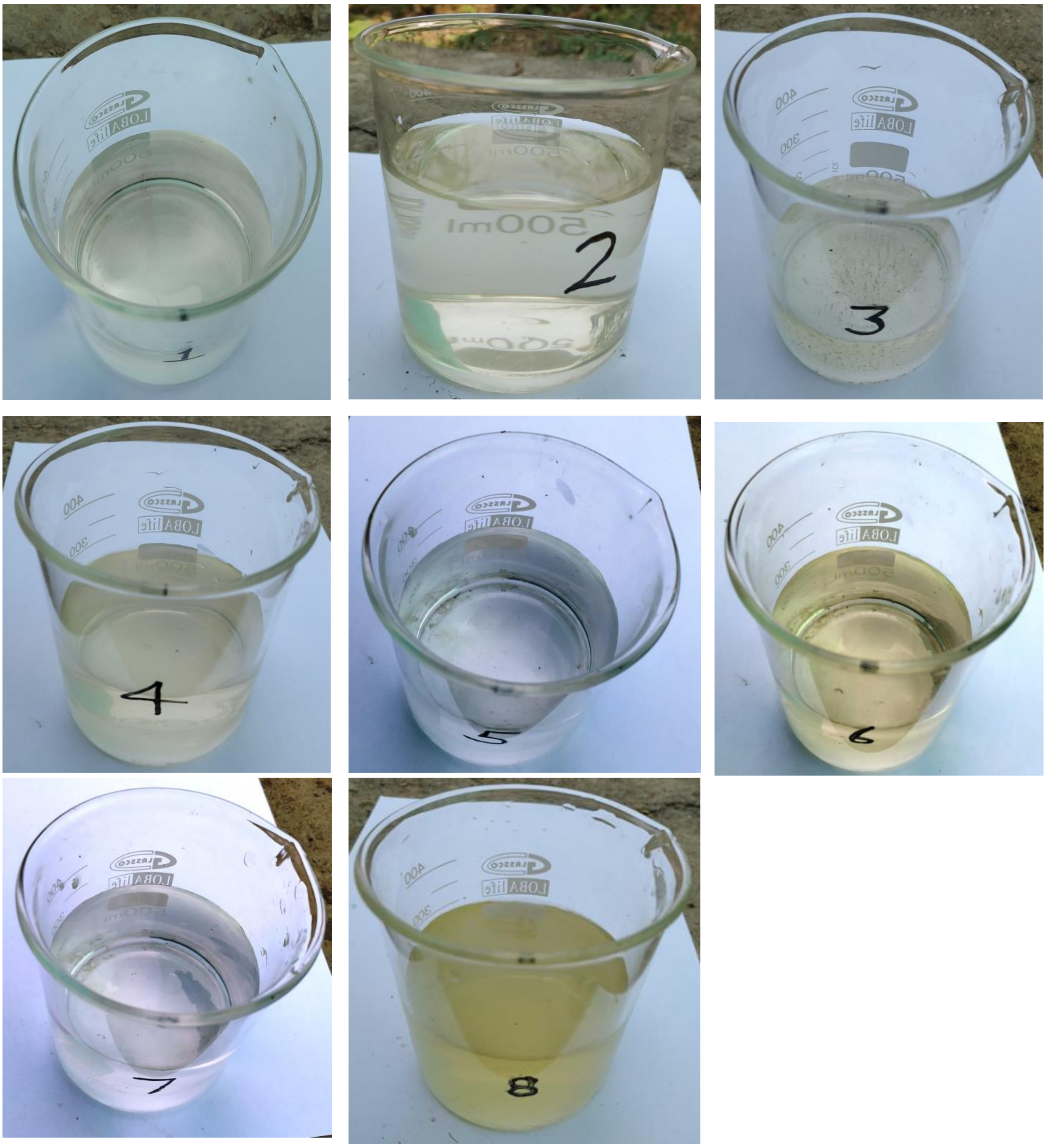
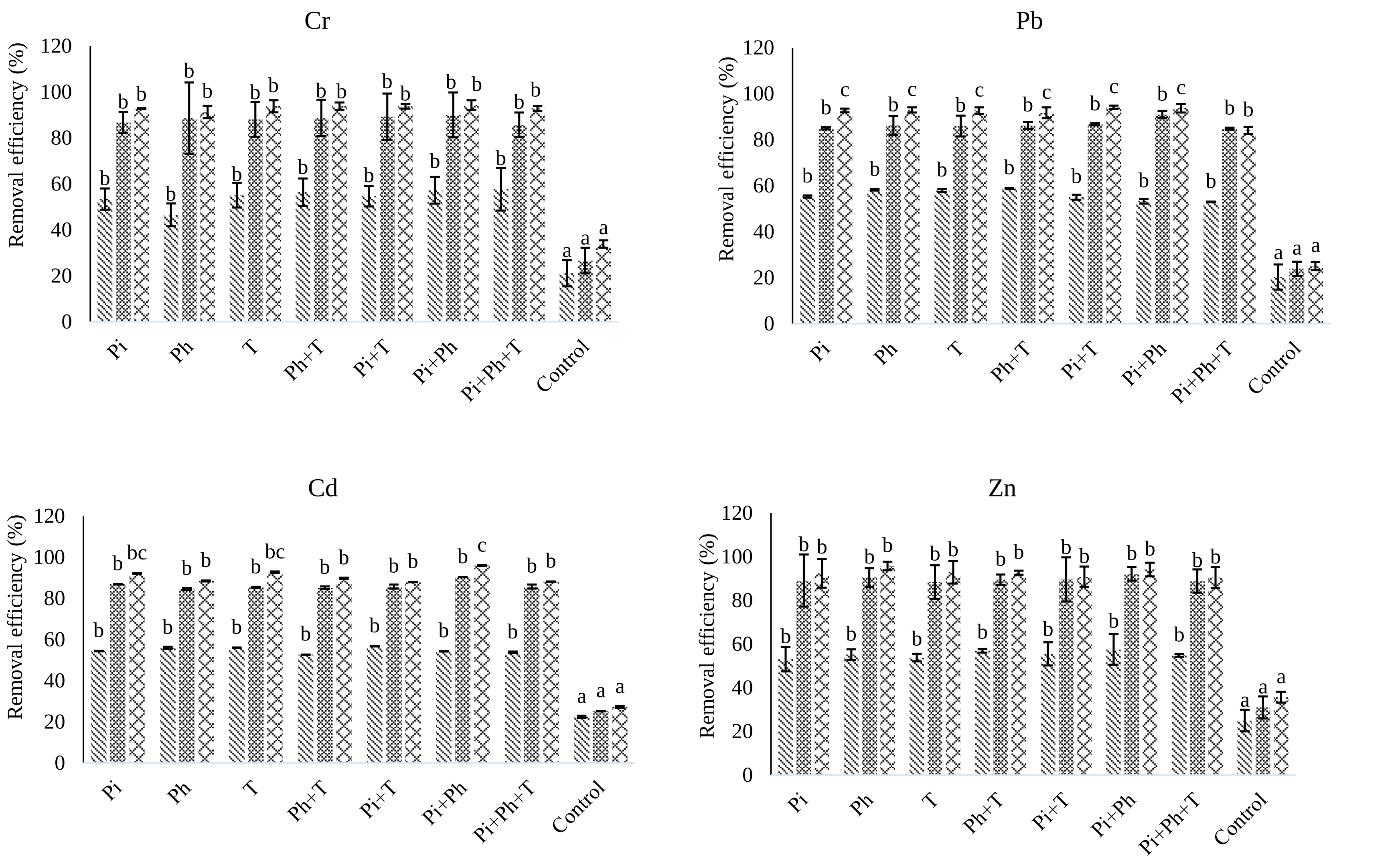


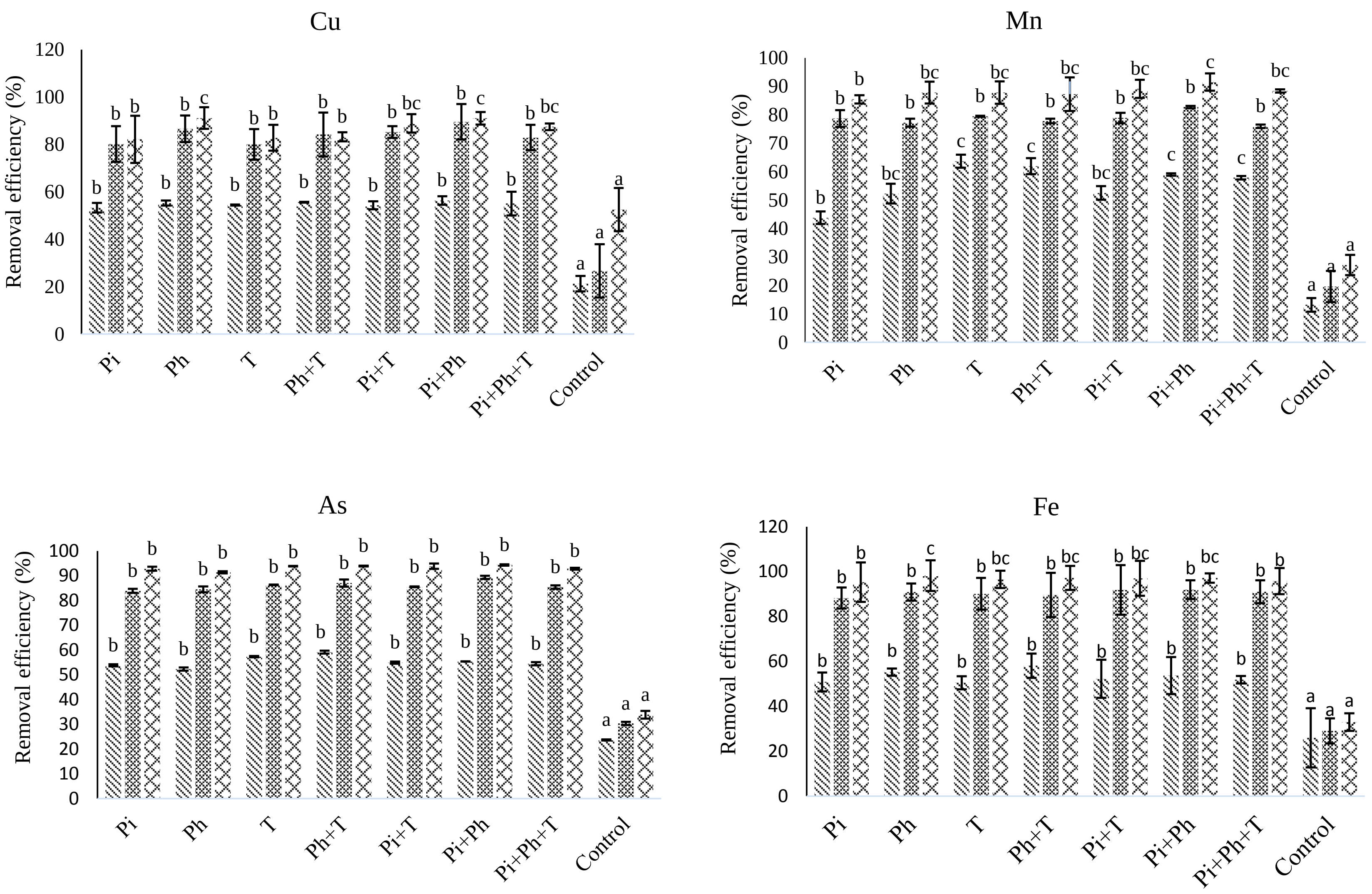
Fig. 3.6 Visual comparison of effluent quality between different treatment units. Number from 1to 8 stands for Pi, Ph, T, Ph+T, Pi+Ph, Pi+T, Pi+Ph+T and Control unit respectively

3.3.3 HMs removal by different CWM units

In CWMs, removal of HMs is done through several processes such as macrophytic uptake, adsorption, precipitation, ion exchange, sedimentation, oxidation and reduction and flocculation etc. Planted macrophytes exhibited vital role in the exclusion of HMs by the phytoaccumulation, phytovolatilization and phytostabilization. From one-way ANOVA analysis ($p < 0.05$), the significant variances between removal efficacies of selected HMs with respect to HRTs and CWM units were observed (Fig. 3.7). The performance of CWM units for all HMs considered ranged from 43.80 to 63.67% for 3 d, 75.92 to 92.07% for 7 d and 82.17 to 98.58 % for 14 d HRT respectively. The removal of Cr was recorded maximum in CWM units Pi+Ph followed by Pi+Ph+T at 3 d and again by Pi+Ph at 7 and 14 d respectively. For Cd, the higher removal efficacy was revealed in CWM unit Pi+T at 3 d and Pi+Ph at 7 and 14 d respectively. However, the maximum elimination rate of Pb was recorded in units Ph+T, Pi+Ph and Pi+T for all three HRTs respectively. The highest elimination rate for Zn was exhibited by unit Pi+Ph and Ph at all three HRTs. The similar results were also observed for Cu by the same CWM unit. However, the maximum elimination rate of Mn was shown by unit T at 3 d and Pi+Ph at 7 and 14 d respectively. Removal of Fe was found maximum in the treatment units Ph+T and Pi+Ph at 3 and 7 d respectively and Ph (98.14%) at 14 d. Elimination of As and Ni also displayed similar results amongst various treatment units with maximum efficiency by units Pi+Ph, Ph+T and Pi+T. From the above results, it is evaluated that the CWM unit having *Pistia stratiotes* and *Phragmites karka* together achieved excellent results for most of the HMs at dissimilar HRTs. The elimination percentage of all selected HMs at 7 and 14 d HRTs reached up to 92% and 98% individually. The excellent HMs elimination capacity of designed treatment units may be due to the aerobic environment within them through which HM cations co-precipitated into hydroxides, oxyhydroxides and ferric oxides (Vymazal and Brezinova, 2016). Removal of HMs also comprises of numerous chemical responses such as complexation and precipitation amongst the metals and ions released by the microbial populations (e.g., CO_3^{2-}), substrate materials (e.g., CO_3^{2-} and OH^-) and macrophytes (e.g., organic acids) (Nuttall and Younger, 2000; Liu et al., 2007; Di Luca et al., 2011; Wang et al., 2020). In this study, the elimination of all the selected HMs was found almost constant throughout the year with standard deviations less than 15.61% which shows the stable performance of CWM units.



Cr
Pb
Cd
Zn
Removal efficiency (%)
0
20
40
60
80
100
120
Pi
Ph
T
Ph+T
Pi+T
Pi+Ph
Pi+Ph+T
Control


Cu
Removal efficiency (%)
Pi
Ph
T
Ph+T
Pi+T
Pi+Ph
Pi+Ph+T
Control
Mn
Removal efficiency (%)
Pi
Ph
T
Ph+T
Pi+T
Pi+Ph
Pi+Ph+T
Control
As
Removal efficiency (%)
Pi
Ph
T
Ph+T
Pi+T
Pi+Ph
Pi+Ph+T
Control
Fe
Removal efficiency (%)
Pi
Ph
T
Ph+T
Pi+T
Pi+Ph
Pi+Ph+T
Control

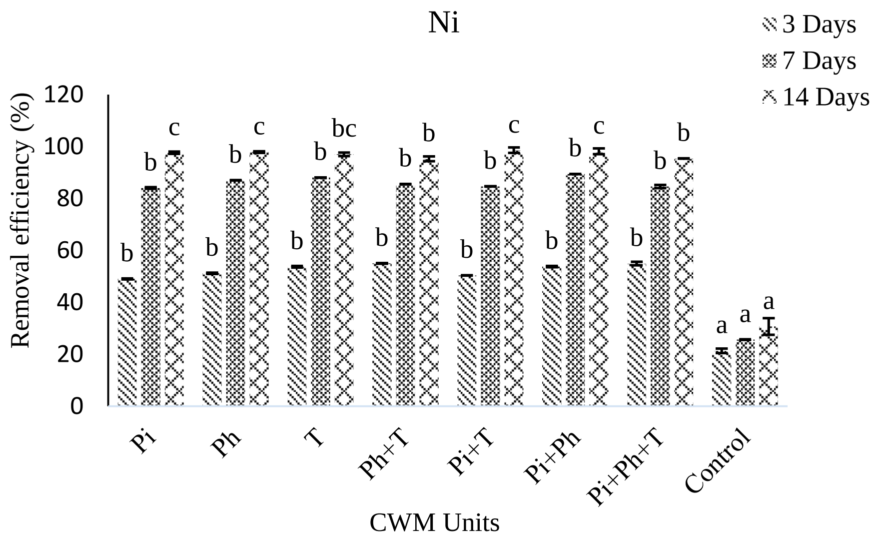


Fig. 3.7 Removal efficacy of various HMs by different CWM units at three HRTs (mean ± SD, n = 12). Various letters (a, b and c) as superscript describe the variance amongst the mean removal efficacies ($p < 0.05$)

3.3.4 Concentration of heavy metals within the root and aerial parts of selected macrophytes

Macrophytes such as *Phragmites sp.* and *Typha sp.* are considered as hyperaccumulators for numerous HMs (Salem et al., 2014). Most of HMs are removed frequently via the absorption through the roots of available macrophytes (Scholz and Hedmark, 2010). Besides, some HMs are translocated into aboveground biomass of the macrophytes (Lu et al., 2013). This may be due to the sequestration of HMs in vacuoles. It is a natural phenomenon of resistance to remove possible toxic effects of HM ions (Shanker et al., 2005). However, numerous ions and acids such as citrate, oxalate, malonate, acetate and organic acids are ejected as root exudates via macrophytes that perform as chelators to bind HM ions and catalyze various reactions (Ryan et al., 2001). Utilization of substrate materials inside CWMs also helps in rhizodeposition of HM ions (Batool and Saleh, 2019). The concentration of HMs in the roots and aerial parts of selected macrophytes together with BCF, TF, ATCF and RCF and substrate have been specified in table 3.4, 3.5 and 3.6 separately. BCF is explained as the ability of macrophytes to buildup HMs from the substrates whereas TF assess the potential and feasibility of selected macrophytes for the phytoremediation process (Wu et al., 2011). It is known that macrophytes with maximum BCF and TF possess great capability for the remediation of HMs in the natural setting (Ndeda and Manohar, 2014). In this study, maximum TF and BCF were exhibited by *Pistia stratiotes* and *Phragmites karka* for

Zn (0.69 and 1.69 respectively). However, maximum RCF and ATCF were observed in *Pistia stratiotes* for Cu and Zn (0.35 and 0.10 respectively). Overall, *Pistia stratiotes* exhibited great potential towards the accumulation of Zn in root as well as aerial parts. It is reported that the concentration of HMs in roots of *Phragmites* was found 10–100 times higher as compared to aboveground biomass (Vymazal, 2016). Though, the elevated accumulation of HMs such as Fe in *Typha latifolia* may be because of their aggressive nature for competition amongst other selected macrophytes. CWMs with aerobic settings encourage the formation of hydroxides, oxyhydroxides and oxides of Fe and Mn that may help in the removal of HMs by complexation (Vymazal, 2016). An earlier study advocates that the concentration of HMs at the sediment inlet are found higher as compared to the outlet (Hadad et al., 2006). In the present research work, it is observed that the accumulation of Pb in the substrate material was maximum as compared to other selected HMs. The higher accumulation of Pb may be due to its high adsorption capacity. It is also evaluated in an earlier study that the Pb has elevated adsorption ability with great binding capability as compared to other HMs (Kinraide and Yermiyahu, 2007). The effective removal of Cr and Fe through *Typha latifolia* is revealed in earlier research performed by Mustapha et al. (2018). The accumulation of HMs in macrophytes utilized in CWMs also comprises of several steps; such as translocation via root cortex and epidermis into the xylem and rhizosphere mobilization etc. (Clemens et al., 2002). Numerous earlier studies have stated the importance of macrophytes for the exclusion of HMs within CWMs (Khan et al., 2009; Vymazal, 2016; Batool and Saleh, 2019). It is also described that the bioavailability of HMs has great impact on the elimination (Vymazal and Brezinova, 2016).

Table 3.4 HMs concentration in soil substrate and parts of *Pistia stratiotes* respectively (mean ± SD, n = 12, at 14 d HRT)

HMs	Substrate mg kg^{-1}	Root mg kg^{-1}	Leaf+ Stem mg kg^{-1}	TF	BCF	RCF	ATCF
Cr	0.80 ± 0.1^{a}	0.13 ± 0.02^{a}	0.03 ± 0.01^{a}	0.29 ± 0.05^{b}	0.20 ± 0.06^{a}	0.11 ± 0.02^{b}	0.03 ± 0.002^{a}
Cd	0.83 ± 0.03^{a}	0.10 ± 0.03^{a}	0.03 ± 0.01^{a}	0.32 ± 0.04^{b}	0.15 ± 0.01^{a}	0.09 ± 0.02^{b}	0.03 ± 0.005^{a}
Pb	3.5 ± 0.2^{ab}	1.05 ± 0.09^{ab}	0.21 ± 0.2^{ab}	0.20 ± 0.02^{b}	0.51 ± 0.12^{a}	0.20 ± 0.01^{a}	0.04 ± 0.007^{b}
Zn	11.70 ± 0.9^{b}	2.32 ± 0.65^{b}	1.6 ± 1.1^{ab}	0.69 ± 0.06^{bc}	1.80 ± 0.5^{a}	0.15 ± 0.07^{a}	0.10 ± 0.002^{c}
Cu	1.1 ± 0.3^{a}	0.61 ± 0.04^{a}	0.05 ± 0.1^{a}	0.08 ± 0.4^{a}	0.61 ± 0.05^{b}	0.35 ± 0.05^{a}	0.03 ± 0.001^{a}
As	1.09 ± 0.02^{a}	0.28 ± 0.006^{a}	0.03 ± 0.02^{a}	0.13 ± 0.03^{a}	0.29 ± 0.01^{bc}	0.20 ± 0.05^{b}	0.03 ± 0.004^{a}
Mn	3.05 ± 0.5^{a}	0.49 ± 0.08^{a}	0.05 ± 0.3^{a}	0.12 ± 0.05^{a}	0.22 ± 0.08^{b}	0.14 ± 0.04^{a}	0.02 ± 0.002^{a}
Ni	2.83 ± 0.4^{a}	0.4 ± 0.002^{a}	0.06 ± 0.53^{a}	0.17 ± 0.07^{a}	0.21 ± 0.05^{a}	0.12 ± 0.06^{a}	0.02 ± 0.006^{a}
Fe	12 ± 1.25^{b}	4.09 ± 0.95^{b}	1.09 ± 1.52^{ab}	0.27 ± 0.03^{b}	1.43 ± 0.57^{b}	0.24 ± 0.02^{a}	0.06 ± 0.01^{b}

Different letters as superscript specify the significant variance amongst mean concentrations of HMs ($p < 0.05$).

Table 3.5 HMs concentration in soil substrate and parts of *Phragmites karka* respectively (mean ± SD, n = 12, at 14 d HRT)

HMs	Substrate mg kg^{-1}	Root mg kg^{-1}	Leaf+ Stem mg kg^{-1}	TF	BCF	RCF	ATCF
Cr	1.02 ± 0.01[a]	0.14 ± 0.002[a]	0.01 ± 0.001[ab]	0.11 ± 0.01[a]	0.15 ± 0.01[bc]	0.12 ± 0.03[a]	0.01 ± 0.001[a]
Cd	0.75 ± 0.01[a]	0.21 ± 0.003[a]	0.01 ± 0.001[a]	0.07 ± 0.01[a]	0.30 ± 0.02[ab]	0.19 ± 0.01[b]	0.01 ± 0.002[a]
Pb	4.02 ± 0.05[a]	1.02 ± 0.05[a]	0.03 ± 0.002[a]	0.03 ± 0.02[b]	0.29 ± 0.02[ab]	0.20 ± 0.04[b]	0.01 ± 0.002[a]
Zn	10.21 ± 0.15[ab]	4.05 ± 0.25[ab]	1.19 ± 0.004[ab]	0.32 ± 0.01[ab]	1.69 ± 0.31[d]	0.23 ± 0.01[bc]	0.07 ± 0.001[b]
Cu	1.73 ± 0.3[a]	0.4 ± 0.008[a]	0.1 ± 0.001[a]	0.04 ± 0.01[a]	0.25 ± 0.04[ab]	0.17 ± 0.01[a]	0.01 ± 0.003[a]
As	1.03 ± 0.05[a]	0.25 ± 0.006[a]	0.02 ± 0.001[a]	0.08 ± 0.001[b]	0.26 ± 0.04[ab]	0.17 ± 0.02[a]	0.01 ± 0.004[a]
Mn	2.94 ± 0.06[ab]	0.87 ± 0.02[a]	0.07± 0.002[a]	0.09 ± 0.02[bc]	0.37 ± 0.07[cd]	0.22 ± 0.002[bc]	0.02 ± 0.005[a]
Ni	2.80 ± 0.04[a]	0.5 ± 0.04[a]	0.03 ± 0.001[a]	0.06 ± 0.01[a]	0.21 ± 0.06[b]	0.14 ± 0.05[a]	0.01 ± 0.001[a]
Fe	14.07 ± 0.15[a]	3.02 ± 0.85[a]	1.50 ± 0.02[a]	0.50 ± 0.05[cd]	1.71 ± 0.2[d]	0.15 ± 0.12[a]	0.07 ± 0.002[b]

Different letters as superscript specify the significant variance amongst mean concentrations of HMs ($p < 0.05$).

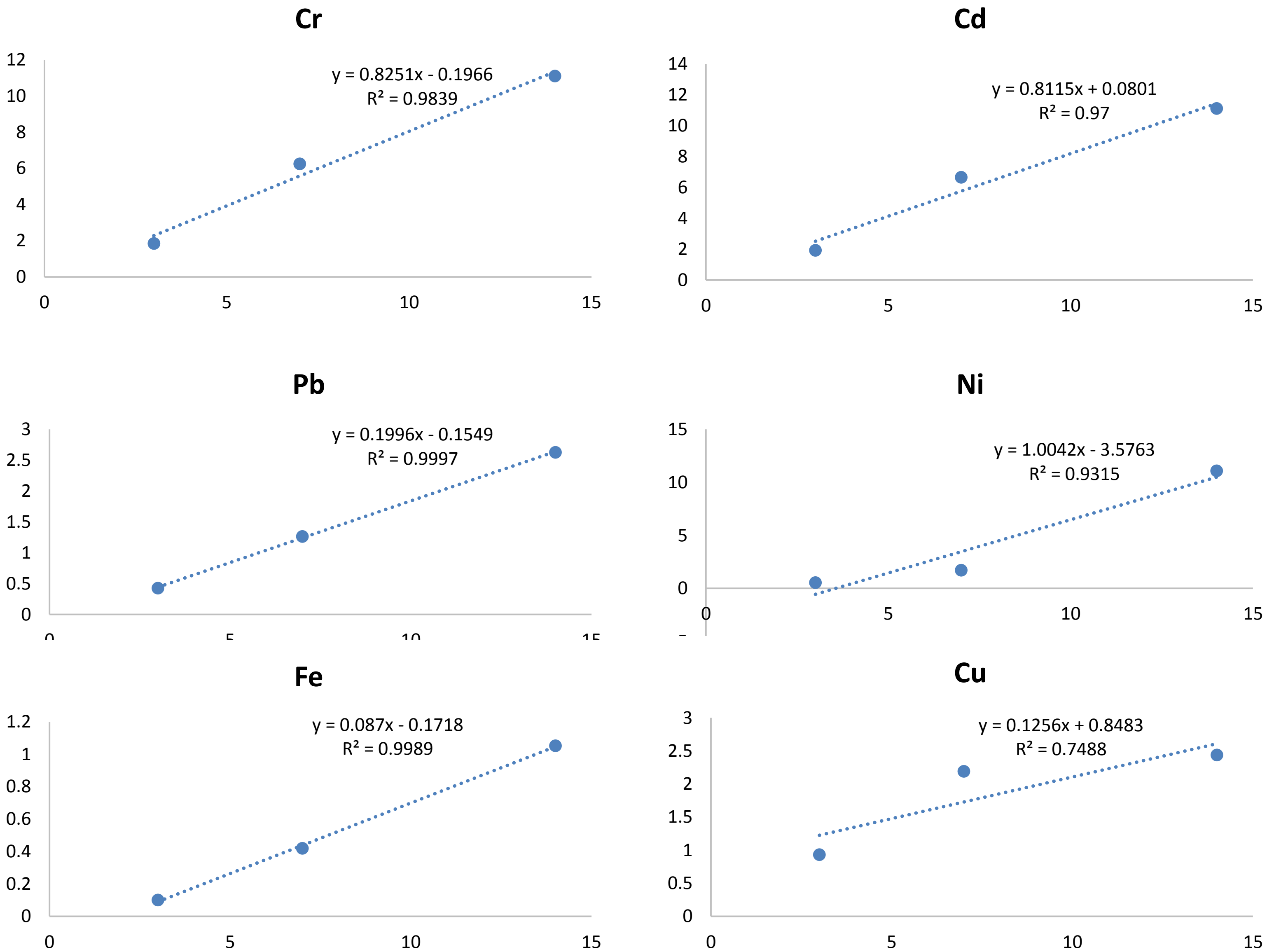
Table 3.6 HMs concentration in soil substrate and parts of *Typha latifolia* respectively (mean ± SD, n = 12, at 14 d HRT)

HMs	Substrate mg kg^{-1}	Root mg kg^{-1}	Leaf+ Stem mg kg^{-1}	TF	BCF	RCF	ATCF
Cr	1 ± 0.02^{a}	0.04 ± 0.006^{a}	0.02 ± 0.001^{a}	0.34 ± 0.02^{a}	0.12 ± 0.01^{a}	0.08 ± 0.01^{a}	0.03 ± 0.02^{a}
Cd	0.7 ± 0.01^{a}	0.06 ± 0.005^{a}	0.03 ± 0.02^{a}	0.55 ± 0.04^{b}	0.13 ± 0.01^{bc}	0.07 ± 0.01^{a}	0.04 ± 0.006^{a}
Pb	3.25 ± 0.2^{a}	0.72 ± 0.009^{a}	0.04 ± 0.02^{ab}	0.10 ± 0.07^{a}	0.31 ± 0.01^{c}	0.18 ± 0.04^{a}	0.02 ± 0.005^{a}
Zn	11.59 ± 0.2^{ab}	3.02 ± 0.12^{b}	1.03 ± 0.05^{b}	0.32 ± 0.02^{a}	1.27 ± 0.51^{cd}	0.18 ± 0.02^{b}	0.06 ± 0.004^{b}
Cu	1.6 ± 0.03^{a}	0.14 ± 0.004^{a}	0.03 ± 0.001^{a}	0.17 ± 0.07^{a}	0.16 ± 0.03^{b}	0.10 ± 0.01^{a}	0.02 ± 0.003^{b}
As	0.69 ± 0.05^{a}	0.24 ± 0.006^{a}	0.02 ± 0.02^{a}	0.14 ± 0.06^{a}	0.24 ± 0.04^{a}	0.17 ± 0.02^{b}	0.02 ± 0.001^{a}
Mn	2.31 ± 0.4^{a}	0.90 ± 0.001^{a}	0.06 ± 0.02^{a}	0.11 ± 0.32^{a}	0.40 ± 0.02^{bc}	0.23 ± 0.05^{bc}	0.02 ± 0.002^{a}
Ni	2.50 ± 0.6^{a}	0.61 ± 0.005^{a}	0.80 ± 0.03^{a}	0.31 ± 0.08^{a}	0.41 ± 0.02^{cd}	0.17 ± 0.01^{a}	0.05 ± 0.01^{a}
Fe	12.17 ± 0.8^{ab}	3.22 ± 0.85^{ab}	1.39 ± 0.08^{b}	0.56 ± 0.01b^{c}	2.00 ± 0.62^{b}	0.16 ± 0.04^{a}	0.09 ± 0.03^{b}

Different letters as superscript specify the significant variance amongst mean concentrations of HMs ($p < 0.05$).

3.3.5 Removal rate

The kinetics of heavy metals removal were evaluated to determine the degree of metal removal with respect to time through various macrophytes (Titah et al., 2019). The exclusion kinetics of heavy metals in this research work fitted with a second-order kinetic model as expressed in fig. 3.8 (R^2= 0.9). The value of decay constants for several metals concerning with different retention times in single as well as mixed CWM units, have been shown in table 3.7 along with their initial and final concentrations. There is a significant difference between the value of decay constant with respect to treatment time. Initially, the rate of reaction for metals removal was higher (for 3 and 7 days) and then slowed down at the end of treatment cycle for the majority of metals in several CWM units. This is due to the fast preliminary adsorption in the beginning, followed by a slow macrophytic uptake. It is known that in CWs, early adsorption is an effective removal mechanism for HMs (Zhang et al., 2020). The relative higher value of decay constant was observed for Cr, Cd and Mn throughout the experiment. With respect to CWM units, the Pi+Ph showed higher removal constant as compared to others for most of the metals. A similar pattern for HMs removal has been described earlier (Luca et al., 2011; Sekomo et al., 2012) in which HMs, mainly Pb, Zn, Cu and Cd from wastewater, were adsorbed primarily by substrate material. In the present work, it is observed that about 50 to 70% of HMs concentrations were removed by adsorption in substrate materials during the initial 3 days. However, the contribution of macrophytic uptake to the overall removal of heavy metals was relatively small (approximately 5-15%).


Cr
y = 0.8251x - 0.1966
R² = 0.9839
Cd
y = 0.8115x + 0.0801
R² = 0.97
Pb
y = 0.1996x - 0.1549
R² = 0.9997
Ni
y = 1.0042x - 3.5763
R² = 0.9315
Fe
y = 0.087x - 0.1718
R² = 0.9989
Cu
y = 0.1256x + 0.8483
R² = 0.7488

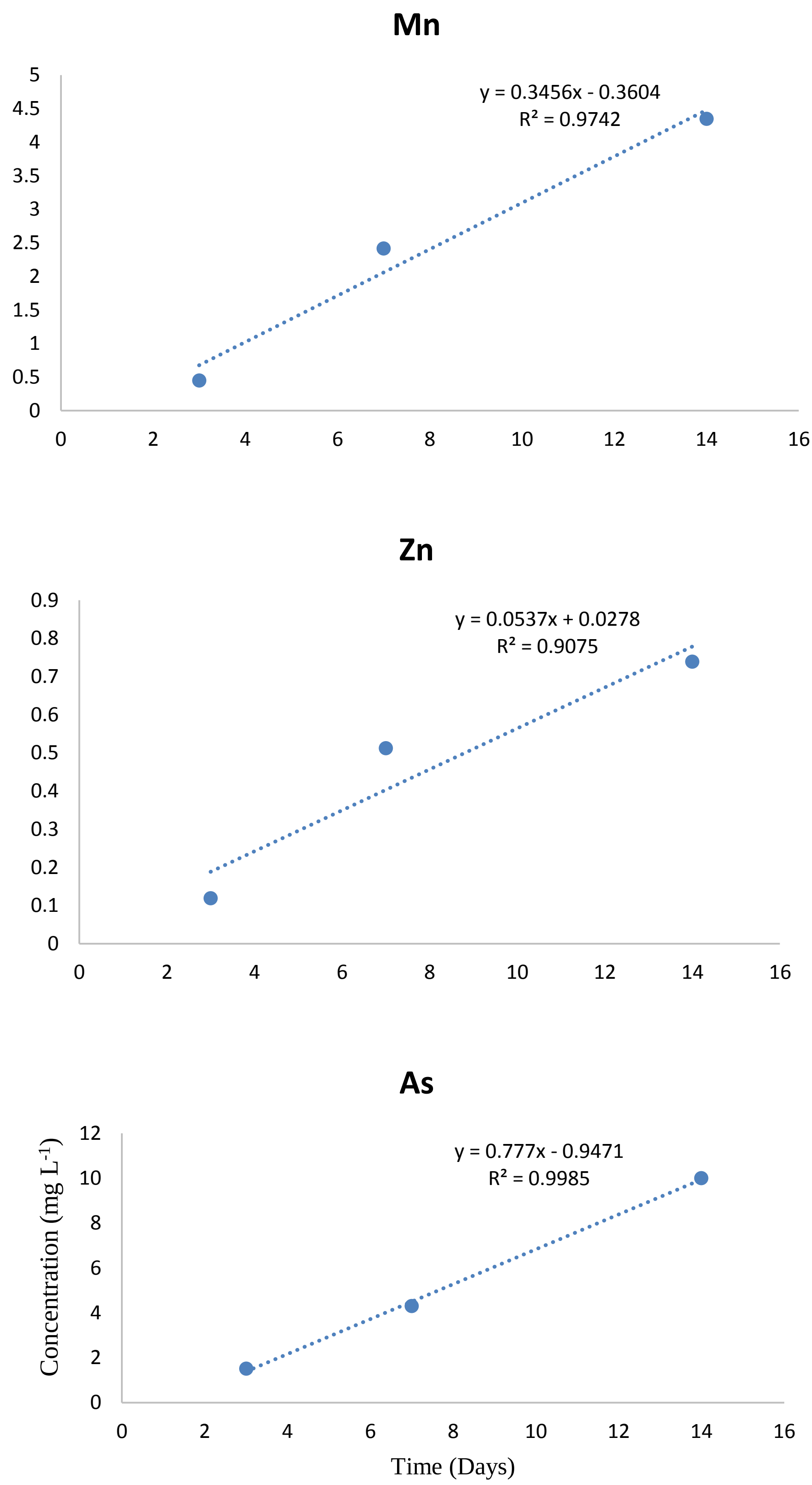


Fig. 3.8 Best fit graphs of concentration Vs time for pseudo second order kinetic model used for different HMs

Table 3.7 HMs removal rate by several CWM units with their initial (A_0) and final (A) concentrations at three HRTs (mean, n = 12)

	Pi									**Ph**								
	Cr	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**	**Cr**	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**
	mg/L																	
A_0	1.21	1.13	5.23	17.85	2.3	1.44	3.96	3.6	20.9	1.21	1.13	5.34	17.85	2.3	1.44	3.96	3.6	20.9
$A_{3\ days}$	0.54	0.52	2.34	8.78	1.28	0.80	2.67	1.86	9.16	0.65	0.50	2.19	7.79	1.50	0.22	2.33	1.79	9.04
$A_{7\ days}$	0.16	0.15	0.79	1.95	0.46	0.23	0.41	0.58	2.39	0.14	0.17	0.71	1.69	0.31	0.12	0.29	0.52	1.84
$A_{14\ days}$	0.09	0.09	0.38	1.35	0.41	0.10	0.23	0.09	0.95	0.11	0.13	0.37	0.76	0.20	0.22	0.16	0.07	0.38
$k_{3\ days}$	0.34^{d}	0.35^{d}	0.08^{d}	0.02^{bc}	0.12^{a}	0.19^{a}	0.04^{a}	0.009^{b}	0.02^{b}	0.24^{b}	0.37^{e}	0.09^{e}	0.02^{bc}	0.09^{a}	0.22^{a}	0.06^{a}	0.009^{b}	0.02^{b}
$k_{7\ days}$	0.77^{c}	0.83^{d}	0.15^{bc}	0.07^{b}	0.25^{a}	0.26^{b}	0.31^{a}	0.02^{b}	0.05^{b}	0.91^{e}	0.71^{b}	0.17^{d}	0.08^{b}	0.40^{a}	0.29^{b}	0.45^{ab}	0.02^{bc}	0.07^{cd}
$k_{14\ days}$	0.74^{c}	0.74^{d}	0.17^{c}	0.05^{b}	0.14^{a}	0.83^{b}	0.20^{a}	0.08^{bcd}	0.07^{b}	0.62^{b}	0.48^{b}	0.18^{cd}	0.09^{c}	0.32^{d}	0.53^{a}	0.43^{a}	0.1^{cd}	0.19^{d}

	T									**Ph+T**								
	Cr	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**	**Cr**	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**
	mg/L																	
A_0	1.21	1.13	5.34	17.85	2.3	1.44	3.96	3.6	20.9	1.21	1.13	5.23	17.85	2.3	1.44	3.96	3.6	20.9
$A_{3\ days}$	0.55	0.50	2.20	8.32	1.28	0.62	2.15	1.69	9.99	0.53	0.52	2.16	8.36	1.18	0.59	1.52	1.65	8.46
$A_{7\ days}$	0.15	0.17	0.73	2.08	0.46	0.20	0.11	0.43	2	0.14	0.15	0.72	1.88	0.41	0.19	0.08	0.53	2.10
$A_{14\ days}$	0.08	0.08	0.39	1.27	0.40	0.08	0.05	0.11	0.70	0.07	0.09	0.43	1.31	0.36	0.08	0.04	0.17	0.58
$k_{3\ days}$	0.34^{cd}	0.38^{e}	0.09^{e}	0.02^{bc}	0.12^{a}	0.28^{a}	0.07^{a}	0.009^{b}	0.02^{b}	0.35^{e}	0.35^{b}	0.09^{e}	0.02^{bc}	0.14^{a}	0.22^{a}	0.15^{b}	0.11^{b}	0.02^{b}
$k_{7\ days}$	0.87^{d}	0.73^{bc}	0.17^{cd}	0.06^{b}	0.25^{b}	0.35^{b}	1.31^{c}	0.02^{cd}	0.07^{c}	0.93^{e}	0.83^{bc}	0.17^{d}	0.07^{b}	0.33^{b}	0.33^{b}	1.83^{d}	0.02^{bc}	0.06^{bc}
$k_{14\ days}$	0.89^{d}	0.79^{e}	0.17^{c}	0.05^{b}	0.15^{b}	0.76^{b}	1.33^{a}	0.06^{bc}	0.10b^{c}	0.9^{d}	0.74^{c}	0.15^{c}	0.05^{b}	0.15^{b}	0.76^{b}	3.19^{a}	0.04^{b}	0.12^{c}

	Pi +T									Pi+ Ph								
	Cr	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**	**Cr**	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**
									mg/L									
A_0	1.21	1.13	5.34	17.85	2.3	1.44	3.96	3.6	20.9	1.21	1.13	5.34	17.85	2.3	1.44	3.96	3.6	20.9
$A_{3\ days}$	0.55	0.49	2.37	8.34	1.51	0.65	1.30	1.82	9.63	0.52	0.52	2.49	7.18	1.03	0.64	1.19	1.69	9.36
$A_{7\ days}$	0.13	0.16	0.70	1.86	0.34	0.21	0.36	0.56	1.65	0.12	0.11	0.48	1.42	0.24	0.15	0.16	0.38	1.63
$A_{14\ days}$	0.08	0.14	0.31	1.65	0.26	0.09	0.11	0.05	0.61	0.07	0.05	0.33	1.05	0.21	0.08	0.04	0.07	0.58
$k_{3\ days}$	0.33^{c}	0.39^{f}	0.08^{cd}	0.02^{bc}	0.09^{a}	0.31^{a}	0.17^{b}	0.008^{b}	0.02^{b}	0.37^{f}	0.35^{d}	0.07^{b}	0.03^{c}	0.18^{a}	0.36^{a}	0.20^{b}	0.009^{b}	0.02^{b}
$k_{7\ days}$	0.98^{f}	0.76^{c}	0.18^{d}	0.08^{b}	0.36^{b}	0.31^{b}	0.37^{a}	$0.02b^{c}$	0.08^{d}	1.06^{g}	1.17^{e}	0.27^{e}	0.09^{b}	0.55^{c}	0.44^{b}	0.91^{cd}	0.03^{d}	0.08^{d}
$k_{14\ days}$	0.89^{d}	0.46^{b}	0.22^{e}	0.04^{b}	0.25^{cd}	0.76^{b}	0.65^{a}	0.14^{e}	0.11^{bc}	0.97^{e}	1.48^{f}	0.20^{de}	0.07^{bc}	0.32^{d}	0.96^{b}	1.62^{a}	0.11^{d}	0.12^{c}

	Pi+ Ph+ T									Control								
	Cr	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**	**Cr**	**Cd**	**Pb**	**Zn**	**Cu**	**As**	**Mn**	**Ni**	**Fe**
									mg/L									
A_0	1.21	1.13	5.34	17.85	2.3	1.44	3.96	3.6	20.9	1.21	1.13	5.34	17.85	2.3	1.44	3.96	3.6	20.9
$A_{3\ days}$	0.51	0.52	2.41	9.32	1.31	0.66	2.24	1.61	9.73	0.96	0.88	4.14	14.11	193	1.10	3.11	2.88	14.95
$A_{7\ days}$	0.17	0.16	0.80	1.99	0.39	0.21	0.56	0.56	1.81	0.89	0.85	3.98	12.32	1.69	1	2.46	2.72	14.33
$A_{14\ days}$	0.09	0.13	0.84	1.70	0.29	0.10	0.33	0.17	0.86	0.8	0.82	0.92	11.49	1.09	0.95	1.93	2.54	13.53
$k_{3\ days}$	0.37^{f}	0.34^{c}	0.07^{c}	0.02^{b}	0.12^{a}	0.30^{a}	0.06^{a}	0.009^{b}	0.02^{b}	0.07^{a}	0.08^{a}	0.02^{a}	0.01^{a}	0.03^{a}	0.06^{a}	0.03^{a}	0.002^{a}	0.01^{a}
$k_{7\ days}$	0.70^{b}	0.76^{c}	0.15^{b}	0.06^{b}	0.31^{b}	0.30^{b}	0.22^{a}	$0.02b^{c}$	0.07^{cd}	0.04^{a}	0.04^{a}	0.01^{a}	0.004^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.001^{a}	0.003^{a}
$k_{14\ days}$	0.76^{c}	0.47^{b}	0.07^{b}	0.04^{b}	0.22^{c}	0.74^{b}	0.20^{a}	0.04^{b}	0.08^{bc}	0.03^{a}	0.02^{a}	0.005^{a}	0.002^{a}	0.03^{a}	0.03^{a}	0.02^{a}	0.001^{a}	0.002^{a}

Different letters as superscript represent the significant variance amongst the mean removal rates for various CWM units at three HRTs ($p < 0.05$). The unit of k is mg L^{-1} d^{-1}.

3.3.6 Correlation studies amongst removal efficacy of HMs and other pollutants

The relationship between HMs removal efficacies and with various other selected water quality parameters for most effective CWM unit (Pi+Ph) has been evaluated at all three HRTs. The correlation results revealed significant differences depending upon HMs, HRTs and other selected parameters at $p < 0.05$. The most of the HMs exhibited significant positive correlation with As. Some HM pair and other contaminants showed significant positive correlation with each other, these were Cr and BOD, Zn with NH_4^+-N and Ni with Cu, SRP, NH_4^+-N and NO_3^--N. Nevertheless, the significant negative correlation was recorded among Cd and As with NO_2^--N and Zn and Cd with BOD, TP and SRP. The parallel correlation outcomes were also revealed in a study described by Zhang et al. (2020) for the treatment of mixed domestic -industrial wastewater via hybrid CWs and by Mishra and Kumar, (2021) for River water. The positive correlations among some HMs may demonstrate that they may have close association, similar accumulation behaviors or derive from the same pollution sources (Titilawo et al., 2018; Agoro et al., 2020). In the present study, both NH_4^+–N and NO_3^-–N expressed positive correlations with majority of HMs. It is reported that concentration of NH_4^+–N can dominate the elimination of heavy metals by macrophytes within CWs (Yin et al., 2018). Nevertheless, associated interaction mechanisms of NH_4^+–N and HMs are not fully explained yet. Furthermore, concentration of Cd could alleviate the negative impact of NH_4^+–N on the growth of macrophytes (Cui et al., 2021). The negative correlation of BOD with majority of the HMs suggests the inhibition of growth and activities of hetero and autotrophic microorganisms may be due to the HM toxicity. High concentration of HMs can reduce the oxidation capabilities and several biochemical activities of these microorganisms resulting in deteriorated microbial biomass and diversity (Wang et al., 2018; Bhat et al., 2020). However, the negative correlation of phosphorus with metals such as Cr and As may be due to the structural analogue that helps in mitigation of HM toxicity. Phosphate and arsenate also share a common transportation pathway via roots of the macrophytes (Sayantan, 2017; Sayantan and Das, 2020).

4. Conclusion

The major objective towards conducting this study was to evaluate the HMs removal kinetics together with treatment efficacy with several other wastewater pollutants in various CWM units at different HRTs. The removal efficacies for all selected HMs as well as other contaminants showed significant variability depending upon CWM units and HRTs. The CWM unit Pi+Ph designed using *Pistia stratiotes* and *Phragmites karka* achieved maximum removal efficiency for most of the contaminants including HMs. It also acquired the maximum DO as compared to others. The removal percentage for all selected HMs in eight CWM units extended from 43.80 to 63.67%, 75.92 to 92.07% and 82.17 to 98.58 % for 3, 7 and 14 d HRTs respectively. However, the higher value of decay constants was recorded for Cr, Cd and Mn respectively. In this study, maximum TF and BCF were expressed by *Pistia stratiotes* and *Phragmites karka* for Zn (0.69 and 1.69 respectively). However, the maximum RCF and ATCF were observed in *Pistia stratiotes* for Cu (0.35) and Zn (0.10) respectively. Overall, the *Pistia stratiotes* exhibited great potential towards the accumulation of Zn in roots as well as in aerial parts. The correlation results for most effective CWM unit between HMs removal efficacies and several other parameters displayed significant differences with respect to type of pollutants and HRT. All selected HMs exhibited significant positive correlation with As. In addition to this, several pairs also exhibited significant positive association with each other, these were Cr and BOD, Zn with NH_4^+-N and Ni with Cu, SRP, NH_4^+-N and NO_3^--N respectively.

Chapter 4

Interspecific competition and their impacts on the growth of macrophytes and pollutants removal

4.1 Introduction

Macrophytes that have approximately the same growth patterns and share unique niche provisions can coexist in a similar environment for the same time period (Chesson, 2000; Warren et al., 2019). Interspecific competition mostly for space, light and nutrients amongst various macrophytes is considered very crucial to define vegetation cover of wetland systems (Connolly et al., 2001; Gioria and Osborne, 2014; Kankanamge and Kodithuwakku, 2017). Removal of nutrients via macrophytes in competitive settings possesses great significance towards the impacts on the performance of CWMs (Zheng et al., 2016). It is reported that CWMs having more than one species of macrophyte are less susceptible to periodic oscillations and are considered as more efficient as compared to CWM with single macrophyte (Liang et al., 2011; Bencsik et al., 2014; Chang et al., 2014). It is described that macrophytes perform several indirect and direct roles in the treatment process within CWMs, in which, the main part is uptake and assimilation of wastewater nutrients, substrates for the attachment of microbial inhabitants, oxygen and root exudates, reduction of wind velocity, regulation of hydraulics and surface insulation etc. (Cui et al., 2011; Vymazal, 2013b; Boog et al., 2014; Farzi et al., 2017; Hadad et al., 2018). Emergent macrophytes can help in the growth of rich microbial inhabitants by developing dense and deep root assemblages (Jampeetong et al., 2012). The *Typha. spp*., *Phragmites spp.*, *Scirpus* and *Juncus* are the major macrophytic species used in CWMs throughout the world because of their excessive reproduction capabilities and flood-tolerant capacity (Vymazal, 2013a; Al-Isawi et al., 2016). Both *Phragmites* and *Typha* are colonial macrophytes that pose similar morphological traits, like tall branched leaves with rhizomes and roots as belowground structures. They also share parallel habitats and a variety of environmental conditions such as resistance towards saline settings (Miklovic and Galatowitsch, 2005). Usually, both macrophytes exhibited dense and robust growth patterns (Shih and Finkelstein, 2008). The zone of interaction between *Typha latifolia* and *Phragmites karka* is probably characterized by competition mainly for space, however, the growth of one macrophyte disturbs the neighboring plants by spatial dynamics. Thus, the mixed colonies of *Typha latifolia* and *Phragmites karka* indicate a classical model of competitive interactions and display strong competition within CWMs. *Pistia stratiotes* is recognized as a free-floating macrophyte having global distribution mainly in the tropics that needs standing or slow-flowing water with excess nutrients concentration (Dipu et al., 2011). Due to the high variation in competition between several macrophytes including those planted in mixed culture, studies dealing with long-term field-scale applications is still very limited. (Kankanamge and Kodithuwakku, 2017; Amon et al., 2007). Therefore, the main aim of conducting this research work was to measure the interspecific competition and relative growth rate between three macrophytes planted in mixed culture and their influence on the treatment performance of CWM units. Removal of wastewater nutrients by several CWM units was also considered to explain the most suitable macrophytic combination for its effective use. The results are valuable for scheming large-scale mixed planting CWMs that are less vulnerable towards seasonal difference and more efficient in eliminating contaminants.

4.2 Material and Methods

4.2.1 CWM units

All the details regarding the design and operation of CWM units have been provided in the material and methods section under the heading description of CWM units within chapter 3. However, the initial density of macrophytes during this first experiment of this work was 9 plants per CWM unit. For the measurement of competitive value (CV) and relative growth rate (RGR), an additional set of the almost same weight and height/length macrophytes were dried and weighed during the plantation. The height/length of *Phragmites karka* and *Typha latifolia* was 50.8 ± 12.36 and 57.15 ± 9.36 cm respectively. Similarly, the mean individual plant's dry weights of *Phragmites karka, Typha latifolia* and *Pistia stratiotes* at the time of plantation were 2.2, 3.07, and 0.75g respectively. The dry weights of macrophytes were used for the analysis of their growth rate and competitive nature. This experiment was conducted from March 2018 to February 2019 in three successive cycles with raw DW (Table 4.1).

Table 4.1 Characteristics of DW during this experiment (mean ± SD, n= 12)

Characteristics of raw DW	**BOD**	**TP**	**SRP**	**NH_4^+-N**	**NO_3^--N**	**NO_2^--N**
Concentration (mgL^{-1})	108.11 ± 8.53	11.68±3.15	8.09 ± 1.16	25.56 ±8.41	12.38 ± 2.76	4.88 ± 2.08

4.2.2 Macrophytes sampling and analysis

The total number of each macrophyte and their length in specific CWM units were recorded after 30 days intervals to assess the change in their number, total number, length and dominance characteristics. Plant samples were collected from all CWM units, washed with tap water to eradicate associated debris and divided to determine the root length and height. Macrophytes such as *Pistia stratiotes* develop several neonatal plants instead of growing the biomass of the main plant. Consequently, root length, plant height and other parameters related to growth were not measured for *Pistia stratiotes* (Kankanamge and Kodithuwakku, 2017). On contrary generally, most of the macrophytic species exhibited proliferation in their size, hence allowing them to measure growth-related parameters. The other two macrophytes were divided into BGB and AGB and dried in an oven at 103 degrees Celsius for 48 h. The growth responses between selected macrophytes planted in mixed culture were assessed via CV as exhibited in Eq. 15. The CV between macrophytes offers a means to explain competitive interactions amongst the dissimilar macrophytes (Hong et al., 2014).

$$CV = 100(X_2 - X_1)/X_2 \tag{15}$$

Where X_1 and X_2 represent the average dry weights of specific macrophytes grown individually and in mixed culture respectively.

The RGR was estimated according to Eq. 16 as prescribed by Hunt (1982) and Kankanamge and Kodithuwakku. (2017).

$$RGR = (\mathrm{Log}_e\, W_2 - \mathrm{Log}_e\, W_1)/\ (t_2 - t_1) \tag{16}$$

Here, W_1 and W_2 are the average dry weights of macrophytes at times t_1 and t_2 respectively.

The CV and RGR values between the selected macrophytes were assessed at the end of each cycle that is 120-days and the whole experiment was conducted in three consecutive cycles.

4.2.3 Effluent sample collection and their analysis

The sampling and analysis of effluents discharged from different CWM units were done as provided earlier.

4.2.4 Statistical analysis

All the data presented here are exhibited as the mean ± SD. Differences between the means of several growth-related parameters for different macrophytes were studied over one-way ANOVA ($P < 0.05$) analysis.

4.3 Results and Discussion

4.3.1 CV and RGR between selected macrophytes

From the results, it is observed that *Pistia stratiotes* exhibited negative CV with both *Phragmites karka* and *Typha latifolia*. Negative CV of *Pistia stratiotes* with *Phragmites karka* and *Typha latifolia* explained that the overall biomass of *Pistia stratiotes* in monoculture was higher as compared to mixed culture with these macrophytes. Similarly, *Phragmites karka* displayed negative CV with *Typha latifolia* (Table 4.2). It is also due to the higher biomass of *Phragmites karka* in monoculture as compared to the mixed culture. However, the CV remained positive for *Typha latifolia* in all CWM units at all time signifying its overall dominance over two others selected macrophytes (Zheng et al., 2016). Negative CV of *Pistia stratiotes* with *Typha latifolia* and *Phragmites karka* shows that the interspecific competition adversely affected their growth in mixed units (Zheng et al., 2016). From the above results and several previous findings, it is concluded that *Pistia stratiotes* is a weaker competitor against *Typha latifolia*, *Phragmites karka*, and *Eichhornia crassipes* (Agami and Reddy, 1990; Kankanamge and Kodithuwakku, 2017). Nevertheless, it exhibited strong competition with *Limnobium laevigatum* and *Salvinia auriculata* and the competitive ability may be enhanced by providing extra nutrients (Milne et al., 2007). Both *Phragmites karka* and *Typha latifolia* are emergent plants, raising leaves upright over the water surface that limits the accessibility of upcoming sunlight for free-floating *Pistia stratiotes* (Agami and Reddy, 1990). Root biomass per plant of *Pistia stratiotes* is also very less. Correspondingly, *Phragmites karka* also possessed a lower competitive value against *Typha latifolia* due to its dense canopy, aggressive and dominant characteristics that inhibit the growth of neighboring plants in mixed culture (Keddy, 2010). It has the capability to grow vigorously and develop more biomass in nutrient-rich settings as compared to other macrophytes. Therefore, the RGR of *Typha latifolia* was almost two times higher than the *Phragmites karka* in all CWM units. However, *Phragmites spp.* are more frequently used macrophyte for the phytoremediation of almost all types of wastewaters these days (Rezania et al., 2019). Various studies throughout the globe have led to the understanding of competitive behavior of different macrophytes in mixed cultures under varied ecological conditions (Martin and Coetzee, 2014). Agami and Reddy, (1990) conducted a study on competitive interactions among *Pistia stratiotes* and *Eichhornia crassipes* in nutrients rich conditions which exhibited that the high plasticity and dense growth of *Eichhornia crassipes* stressed the growth of *Pistia stratiotes* by limiting the light. *Hydrilla verticillata* in additional nutrient conditions have been also found about seven times stronger competitor when planted with *Vallisneria americana* (Kankanamge and Kodithuwakku, 2017; Van et al., 1999). From the results of several previous studies directing towards the competitive interactions between macrophytes, it is stated that the presence of nutrient and prevailing ecological settings has great influence on the competitive ability of macrophytes (Mony et al., 2007). Together with these, numerous morphological traits, such as leaf shape and size of canopy and shoot are also greatly associated with healthier competition in macrophytes.

Table 4.2 CV and RGR of different macrophytes between various CWM units (mean ± SD, n= 3)

Sr. No	**Unit**	**C. V.**			**RGR ($g^{-1}day^{-1}$)**		**Dominance**
		Pi	**Ph**	**T**	**Ph**	**T**	
1	Pi+Ph	-22.22 ± 3.5^{a}	38.09 ± 2.32^{b}	NA	0.018 ± 0.001^{b}	NA	Pi < Ph
2	Ph+T	NA	- 30 ± 3.52^{a}	23.91 ± 2.25^{a}	0.012 ± 0.002^{ab}	0.02 ± 0.004^{a}	Ph < T
3	Pi+T	-46.66 ± 2.45^{a}	NA	50.70 ± 2.8^{a}	NA	0.026 ± 0.006^{b}	Pi < T
4	Pi+Ph+T	-76 ± 4.12^{a}	- 85.71 ± 3.15^{a}	33.96 ± 3.6^{a}	0.009 ± 0.001^{a}	0.023 ± 0.008^{a}	Pi < Ph < T
5	Ph	NA	NA	NA	0.014 ± 0.003^{ab}	NA	NA
6	T	NA	NA	NA	NA	0.022 ± 0.004^{a}	NA

Various letters as superscripts expressed significant variation between mean values of CV and RGR of macrophytes planted in different CWM units ($p < 0.05$). *NA= Not Applicable

The existence of dissimilar macrophytes together with others put pressure on adjacent macrophytes majorly for light, space and nutrients (Craine and Dubinsky, 2013). Different characters of macrophytes permit them to compete with others to obtain essential resources in the condition of higher versus lower resource accessibility (Martin and Coetzee, 2014). It is described previously that macrophytes grow more rapidly in nutrient-rich conditions and the competition is majorly for upcoming sunlight. Though, in nutrients fewer circumstances, the competition might shift belowground to enhance the root growth and the development of macrophytes becomes slower (Gioria and Osborne, 2014). Several macrophytes can exploit various niches bounded by the parallel environmental settings, confirming the availability of inaccessible nutrients for their neighbors (Evans and Edwards 2001). This capability is expressed by mixed culture of emergent and floating macrophytes as compared to single planting of emergent or floating macrophytes only.

4.3.2 Plant growth analysis

The growth pattern of *Typha latifolia* and *Phragmites karka* was measured after each 30-d interval by measuring the length in their respective CWM units (Fig. 4.1 and 2). The average height, number of macrophytes and the total number of macrophytes after each 30 d were also recorded in all CWM units by counting the number of plants present in a particular CWM unit (Table 4.3). All selected growth parameters were measured for 12 months in three consecutive cycles The results from the analysis of variance exhibited that there is a significant variation in plant height among different single as well as in mixed experimental units. The maximum height of *Typha latifolia* was observed in unit Pi+T and *Phragmites karka* in-unit Pi+Ph, both in combination with *Pistia stratiotes* at all four samplings.

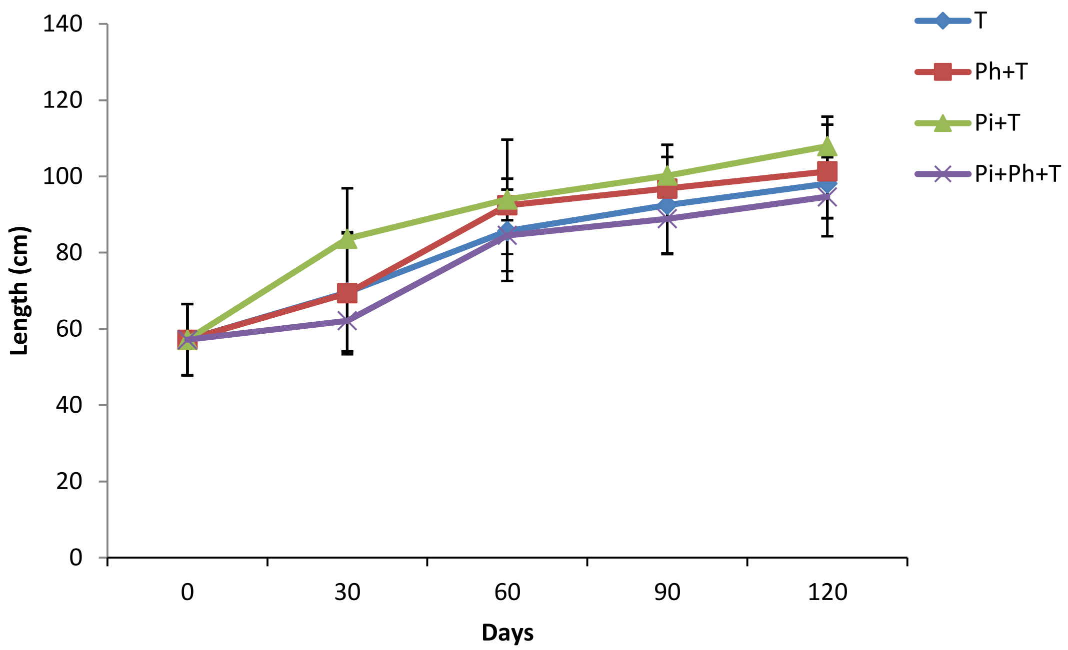


Fig. 4.1 Growth curve of *Typha latifolia* between different CWM units after 30 days interval (mean ± SD, n= 12)

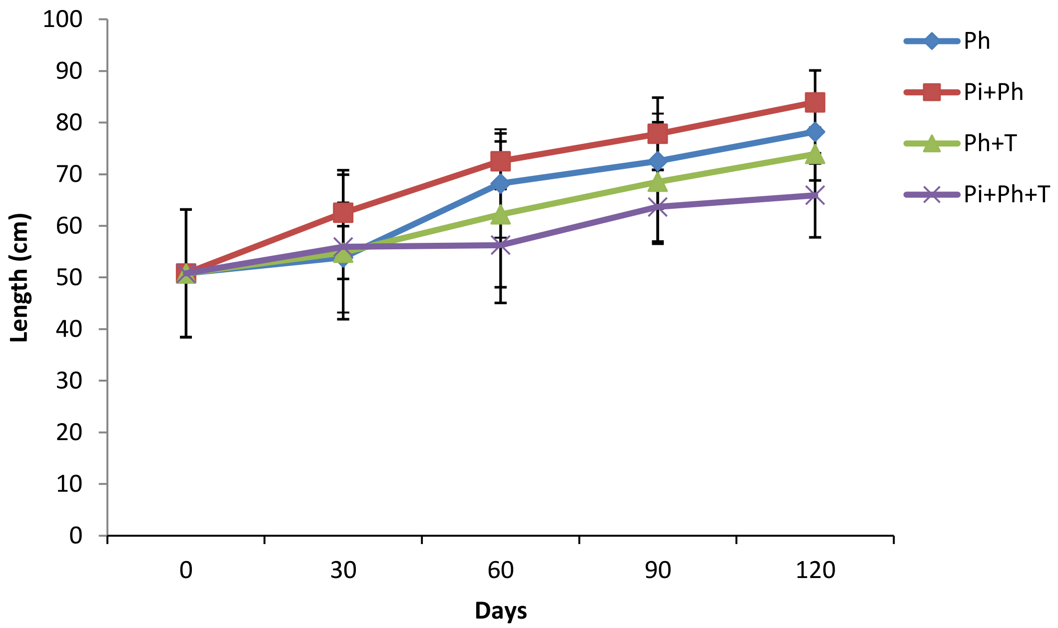


Fig. 4.2 Growth curve of *Phragmites karka* between different CWM units after 30 days interval (mean ± SD, n= 12)

Table 4.3 Number and the total number of macrophytes and their height/ length between different CWM units with respect to time (mean ± SD, n= 3)

Days	Unit	No. of Plants			Total No. of Plants	Plant's height/ Length (cm)	
		Pi	Ph	T		Ph	T
30 d	Pi	16±3	-	-	16±3	-	-
	Ph	-	12±2	-	12±2	53.87±10.65^{a}	-
	T	-	-	15±4	15±4	-	69.61±15.38^{a}
	Pi+Ph	6±2	8±3		14±2	62.52 ± 8.25^{b}	-
	Ph+T	-	9±2	7±2	16±2	54.81 ± 5.12^{a}	69.36±16.02^{a}
	Pi+T	8±3	-	5±1	13±2	-	83.67 ± 13.2^{b}
	Pi+Ph+T	4±2	4±1	9±3	17±3	55.93±14^{a}	62.15±8.10^{a}
60 d	Pi	20±3	-	-	20±3	-	-
	Ph	-	14±4	-	14±4	68.20 ±10.52^{c}	-
	T	-	-	18±3	18±3	-	85.67±6.05^{a}
	Pi+Ph	9±1	12±3	-	21±2	72.54±5.39^{c}	-
	Ph+T	-	12±4	9±3	21±3	62.23±14.12^{b}	92.43±17.25^{ab}
	Pi+T	12±5	-	10±4	22±5	-	93.98±5.46^{b}
	Pi+Ph+T	7±4	8±2	14±2	29±4	56.26±11.20^{a}	84.56±12^{a}
90 d	Pi	31±6	-	-	31±6	-	-
	Ph	-	19±5	-	19±5	72.54±9.23^{bc}	-
	T	-	-	21±4	21±4	-	92.50±12.52^{ab}
	Pi+Ph	17±4	16±3	-	33±4	77.84±7.0^{c}	-
	Ph+T	-	16±5	14±5	30±5	68.54±11.56^{ab}	96.82±8.26^{bc}
	Pi+T	17±7	-	14±4	31±6	-	100.23±8.05^{c}
	Pi+Ph+T	9±4	12±4	20±5	41±4	63.67±7.14^{a}	88.89±9.25^{a}
120 d	Pi	28±4	-	-	28±4	-	-
	Ph	-	24±7	-	24±7	78.23 ± 6.21^{bc}	-
	T	-	-	21±6	21±6	-	98.15±9.20^{a}
	Pi+Ph	22±7	20±4	-	42±6	83.93±6.15^{c}	-
	Ph+T	-	22±7	21±6	43±7	73.92±5.12^{ab}	101.31±12.25^{ab}
	Pi+T	25±6	-	18±3	43±5±	-	107.96 ± 7.69^{b}
	Pi+Ph+T	13±5	15±6	23±4	51±6	65.93±8.15^{a}	94.67±10.32^{a}

*Various alphabets as superscripts signify the significant variances between mean length values of macrophytes for several CWM units at different time intervals ($p < 0.05$).

Growth parameters such as AGB, BGB total biomass and RL of *Typha latifolia* and *Phragmites karka* were recorded at the end of every experimental cycle. Analysis of one-way ANOVA ($p< 0.05$) represents significant variation between several growth parameters with respect to CWM units. The *Phragmites karka* exhibited higher AGB, BGB and total biomass in CWM unit Pi+Ph while the RL was found maximum in-unit Ph. For *Typha Latifolia* the higher values of AGB, BGB and total biomass were found in CWM unit Pi+T, however, the maximum value of RL was recorded in the unit T as compared to other CWM units (Table 4.4).

Table 4.4 Growth-related parameters of macrophytes at the end of the experiment (mean ± SD, n = 3)

Units	Total Biomass (g/ plant)	AGB (g/ plant)	BGB (g/ plant)	RL (cm/ plant)
Pi	5 ± 1^{a}	4.3 ± 0.95^{ab}	0.7 ± 0.02^{a}	5 ± 2.1^{ab}
Ph	24.63 ± 3.65^{a}	11.33 ± 2.5^{a}	13.3 ± 0.8	$28. \pm 2.08^{c}$
T	59.72 ± 4.89^{a}	45.33 ± 3^{a}	14.39 ± 2.0^{ab}	$41.22.66 \pm 0.57^{b}$
Pi+Ph	$(4.5 \pm 0.8^{a}) + (24.95 \pm 8.12^{b})$	$(3 \pm 1.2^{a}) + (12.67 \pm 1.2^{b})$	$(1.5 \pm 0.5^{a}) + (12.28 \pm 1.32^{b})$	$(7 \pm 2.5^{b}) + (22.20 \pm 1.63^{bc})$
Ph+T	$(23.44 \pm 3.32^{a}) + (68.76 \pm 7.21^{a})$	$(10.33 \pm 1.8^{a}) + (47.33 \pm 1.6^{a})$	$(13.13 \pm 0.9^{a}) + (21.43 \pm 3.1^{a})$	$(19.95 \pm 1.3^{a}) + (39.12 \pm 10.44^{b})$
Pi+T	$(4 \pm 0.91^{a}) + (87.91 \pm 12.42^{b})$	$(3 \pm 0.7^{a}) +(74.33 \pm 3.2^{c})$	$(1 \pm 0.05^{a}) +(13.58 \pm 0.7^{ab})$	$(4.6 \pm 2^{a}) + (33.13 \pm 0.5^{a})$
Pi+Ph+T	$(3 \pm 1.2^{b}) + (17.2 \pm 2.15^{a}) + (82.18 \pm 6.31^{b})$	$(2.4 \pm 1^{a}) + (8.67 \pm 1.1^{a}) + (65.33 \pm 2.3^{b})$	$(0.6 \pm 0.002^{a}) + (8.53 \pm 0.6^{a}) + (16.85 \pm 4.5^{b})$	$(4 \pm 1^{b}) + (21.96 \pm 0.8^{ab}) + (36.53 \pm 0.57^{ab})$

*Dissimilar letters as superscripts exhibited significant variances amongst the macrophytes weight and root length planted in various CWM units at $p < 0.05$.

4.3.3 Effect of Interspecific competition on the removal of nutrients

The mixed planting of emergent and free-floating macrophytes within CWMs possesses great removal efficiency for wastewater nutrients through plant uptake as compared to single planting of these macrophytes (Vymazal, 2007; Kumar et al., 2020b). In the present study, it is observed that the CWM unit Pi+Ph displayed maximum removal capability for almost all selected water quality parameters at all HRTs as compared to other CWM units. This is due to the higher nutrient uptake ability that favors the growth of diverse microbes, making available additional O_2 to aerobic bacteria, vigorous growth, regenerating power and less vulnerability to seasonal differences (Leto et al., 2013; Zheng et al., 2016; Rahi et al., 2020). The elimination rate may be further improved by providing intermittent aeration (Liu et al., 2019). The estimation of nutrient concentration in biomass of macrophytes per gram dry weights are considered less significant for nutrient elimination, as it varies critically by the biomass obtained (Zheng et al., 2016). Therefore, it is observed that enhancing the total biomass of macrophytes, plant densities in mixed CWM units and activity of enzymes within substrate media can improve the removal efficacy of nutrients (Leto et al., 2013; Sun et al., 2019). It is stated that the occurrence of microbial populations and removal efficacy of nutrients vary greatly among various macrophytic species (Toscano et al., 2015). It is reported that the uptake of N_2 is associated with the growth of macrophytes within treatment wetlands (Jampeetong et al 2012). However, it does not always mean that macrophytes having higher RGR or superior competitors constantly exhibit maximum elimination rate, especially for nutrients. Consequently, the results of the present study do not follow the results specified by Jampeetong et al. (2012). The author evaluated that the greater uptake ability of nutrients might be a characteristic of the macrophyte and the ability to accumulate additional nutrients concentration is reliant on accessibility as expressed by *Pistia stratiotes* and *Phragmites karka* (Tanner, 1996; Zhang et al., 2020).

4.4 Conclusion

There are noteworthy consequences of interspecific competition on macrophytes growth and nutrient elimination in CWM units established for DW treatment. *Pistia stratiotes* is identified as a weaker competitor against *Phragmites karka* and *Typha latifolia.* The negative CV of *Pistia stratiotes* with *Phragmites karka* and *Typha latifolia* shows that the overall biomass of *Pistia stratiotes* in monoculture is higher as compared to mixed culture with these macrophytes. Similarly, *Phragmites karka* displayed negative CV with *Typha latifolia.* It is also due to the higher biomass of *Phragmites karka* in monoculture as compared to mixed culture. However, the CV remained positive for *Typha latifolia* in all CWM units at all retention time that signified their overall dominance over two others selected macrophytes. Negative CV of *Pistia stratiotes* with *Typha latifolia* and *Phragmites karka* shows that the interspecific competition adversely affected their growth in mixed units. The RGR of

Typha latifolia was always recorded two times higher than *Phragmites karka* in all the CWM units. The zone of interaction between *Phragmites karka* and *Typha latifolia* is characterized by greater competition mainly for space and the progress of one species distresses the adjacent species by spatial dynamics. Therefore, the adjoining colonies of *Phragmites karka* and *Typha latifolia* exhibited a perfect model of competitive interactions. Elimination of wastewater nutrients between different CWM units varied significantly. Removal of all selected water quality parameters was found maximum in mixed planting CWM unit of *Pistia stratiotes* and *Phragmites karka* at most of the time throughout the experiment.

Chapter 5

Activity of different extracellular enzymes within soil substrate and their impacts on the treatment performance of CWM units

5.1 Introduction

Remediation of wastewater through CWMs involves the alteration of complex organic pollutants into simple inorganic constituents (Kong et al. 2009). This has been done via the action of different enzymes within soil substrates and the metabolism of microbes (Kang et al., 1998; Chen et al., 2020). Enzyme activity is considered as a crucial aspect to improving the treatment performance of CWMs (Yan et al., 2018). It is also reported that the activity of different enzymes employed is greatly affected by numerous biotic (microorganisms and macrophytic diversity), edaphic factors (pH, depth, substrate texture and organics and nutrients accessibility,) and predominant climatic settings (Duarte et al., 2008; Kumar et al., 2020c). The supply of additional carbon sources for the improvement of the performance of wetland systems may also alter the enzyme activity to some extent (Shackle et al., 2000). However, the additional source of such extracellular enzymes advances the rate of breakdown of contaminants (Shackle et al., 2006). The deactivated enzymes are also reactivated through the root organization of the macrophytes via oxygenation that are inactivated by tannins and other chemical substances (Neori et al., 2000; Zhang et al., 2007). The biomass of roots of macrophytes has great consequences on the activity of various enzymes within CWMs. Niemi et al. (2005) evaluated 12 soil enzymes that showed a positive correlation among root biomass and activity of enzymes. Later, this study was also supported by Reboreda and Cacador, (2008). Several previous studies stated that the fine root biomass has a great association with elimination rate as compared to total root biomass (Cheng et al., 2009). Consequently, the growth status and activity of roots is critical towards the activity of enzymes. It is reported that macrophytes can influence the activity of several enzymes via releasing extracellular enzymes. They can also alter the diversity of microorganisms and community structure via liberation of root exudates and oxygen within the rhizosphere which have direct effects on enzyme activity. The major objective towards conducting this study was to assess the activity of various enzymes within soil substrate at two depths in different CWM units and their relation with pollutant removal efficacy with respect to time.

5.2 Material and Methods

5.2.1 Description of site

All the details regarding the design and operation of CWM units have been provided in the material and methods section under the sub-heading description of CWM units within chapter 3. However, this experiment was done during October 2018 to September 2019. The characteristics of feeding DW have been provided in table 5.1.

Table 5.1 Average concentration of contaminants in feeding DW (mean ± SD, n=12)

Characteristics of DW	TP	SRP	BOD	NH_4^+-N	NO_3^--N	NO_2^--N
Concentration (mgL^{-1})	11.67±2.85	8.07 ± 2.60	108.11±11.69	25.56 ± 6.26	12.38 ± 4.56	4.88 ± 2.08

5.2.2 Sample collection and analysis

The sampling and analysis of effluents discharged from different CWM units were done as given in chapter 3. However, for the analysis of enzyme activity, the samples of soil from two layers that are top (0-10 cm) and the deeper layer (10-15 cm) were collected from all CWMs every month throughout the experiment. The soil samples were then dried and sieved using 1 mm mesh to eliminate plant debris and other substances present, and then analyzed for the activity of different enzymes. These were Dehydrogenase (DHA), Urease, Phosphatase, Fluorescein Diacetate (FDA) hydrolysis and microbial biomass carbon (MBC).

5.2.3 Enzyme activity assay

5.2.3.1 DHA activity assay

The activity of DHA was estimated by the modified 2,3,5- TTC reduction method as described by Małachowska-Jutsz and Matyja (2019). 5 g of soil sample was placed in tubes and mixed with 0.1 g of $CaCO_3$ and 1.5 ml of deionized water followed by 1 ml of TTC reagent (1%) was added. After that, all the tubes having such a mixture were closed with cotton plugs and placed in an incubator at 30 °C temperature for 24 h for incubation. Finally, the mixture was shifted on filter paper and TPF was separated successively via methanol in a conical flask. The existence of pink color was then read out at 485 nm through spectrophotometer taking methanol as control.

5.2.3.2 Phosphatase activity assay

The activity of phosphatase was quantified by the process prescribed earlier by Schinner and von Mersi (1990). 1 g of soil substrate sample was mixed with 0.25 mL of toluene solution and 4 mL of acetate buffer having pH 5.8. 0.25 mL of above-explained solution was then mixed with 0.115 M pNPP and sited in an incubator for 1 h at 37 ^{0}C. After the incubation time, the reaction was completed by adding 4 mL of 0.5 M NaOH and the development of p-nitrophenol was estimated at 400 nm through a spectrophotometer. The action of phosphatase within soil substrate was represented as µg p-nitrophenol g^{-1} soil h^{-1}.

5.2.3.3 Urease activity assay

The action of urease was assessed by the method prescribed by Klose and Tabatabai (2000). 5 g of soil substrate sample was mixed with 10 ml of phosphate buffer having pH 6.7. After that 0.5 mL toluene and 10 mL of 10% urea solution were added. All the constituents were gently mixed and placed in an incubator for 48 h at 37^{0}C. Afterwards, 20 mL of 1M KCl solution was added and carefully shaken for 30 min and filtered out completely. In 1 mL of filtrate, 9 mL of deionized water was added to make 10 mL of the final solution. After that, in this solution, 1 mL of potassium sodium tartrate, 0.8 mL of Nessler reagent and 4 mL of 1 M NaOH were added sequentially and the final volume was made up to 25 mL by deionized water. Finally, the liberation of NH_4 was measured out through a spectrophotometer at 460 nm. The activity of urease within the soil substrate was exhibited as µg NH_4 g^{-1} soil 24 h^{-1}.

5.2.3.4 Estimation of MBC

Quantification of MBC within soil substrates has been done through the chloroform-fumigation process as described previously (Anderson and Ingram, 1993). Firstly, the samples were dried and sieved to eliminate plant debris via 1 mm mesh. After that, 10 g of soil substrate sample was mixed with 30 ml of chloroform in a conical flask in replicates of three for fumigated extraction (ct1). Then the flasks were placed in a vacuum desiccator until the chloroform vanished totally. The desiccator was then placed in the low light section at 25ºC for five days. Correspondingly, for unfumigated extraction (ct2); 10 g of soil substrate sample was also placed in 125 ml extraction bottles (watertight) and directly extracted using 50 ml of 0.5 M K_2SO_4 with delicately mixing for 30 min. The fumigated soil substrate samples were now taken out. Then, the extracts of soil substrate samples were filtered and 4 ml of filtrate pipetted out. In a flask containing filtrate, 1 ml of 0.0667 M $K_2Cr_2O_7$ and 5 ml of 98% H_2SO_4 were mixed and placed on a heating plate at 150 ºC for 30 min for digestion.

The digested soil samples were then shifted to the second flask and phenanthroline monohydrate (0.3 ml) as an indicator was added. Finally, the samples were titrated by FAS solution and the endpoint was noted as conversion from green to violet red color. The presence of MBC in the soil substrate used in this study was exhibited as µg g^{-1} of soil sample.

5.2.3.5 Estimation of FDA hydrolysis

FDA hydrolysis was estimated by the method discussed earlier by Green et al. (2006). In this process, 1 g of substrate soil sample was mixed with potassium phosphate buffer (15 mL) and FDA solution (0.2 mL) in flasks. The flasks comprising all these reagents were then located in an incubator at 24 ^{O}C for 3 h. After incubation period, 15 mL of extracting mixture (methanol/chloroform as 1:2 v/v) was added. The whole mixture was then centrifugated for 3 min at 10000 rpm. The supernatant was then filtered out and the intensity of FDA hydrolysis was quantified at 490 nm through a spectrophotometer. The FDA hydrolysis within the soil samples was expressed as µg g^{-1} h^{-1}.

5.2.4 Statistical analysis

All the investigational data are presented here as mean ± SD. Analysis of variance among mean enzyme activities within several CWM units corresponding to soil depth and time was performed via one-way analysis of variance ($p < 0.05$). However, the Pearson correlation coefficient was considered to assess the relationships among the enzyme activities and contaminants removal efficacies.

5.3 Results and Discussion

5.3.1 Vertical variation in enzymes activity

The top layers exhibited significantly advanced activity of all enzymes studied in all CWM units and are also significantly different from the deeper layer (Fig. 5.1- 5.5). The significant difference in enzymes action was detected in various CWMs having dissimilar macrophytic combinations. In this study, it was observed that the CWM unit having *Pistia stratiotes* and *Phragmites karka* (Pi+Ph) displayed the maximum activity of enzymes in both layers for all enzymes excluding MBC. However, a higher value of MBC was revealed in the unit having *Typha latifolia* only. Various other CWMs planted with *Phragmites karka* +*Typha latifolia* and *Phragmites karka* only

(Ph+T and Ph) also achieved good results for several enzymes in both layers of soil substrate.

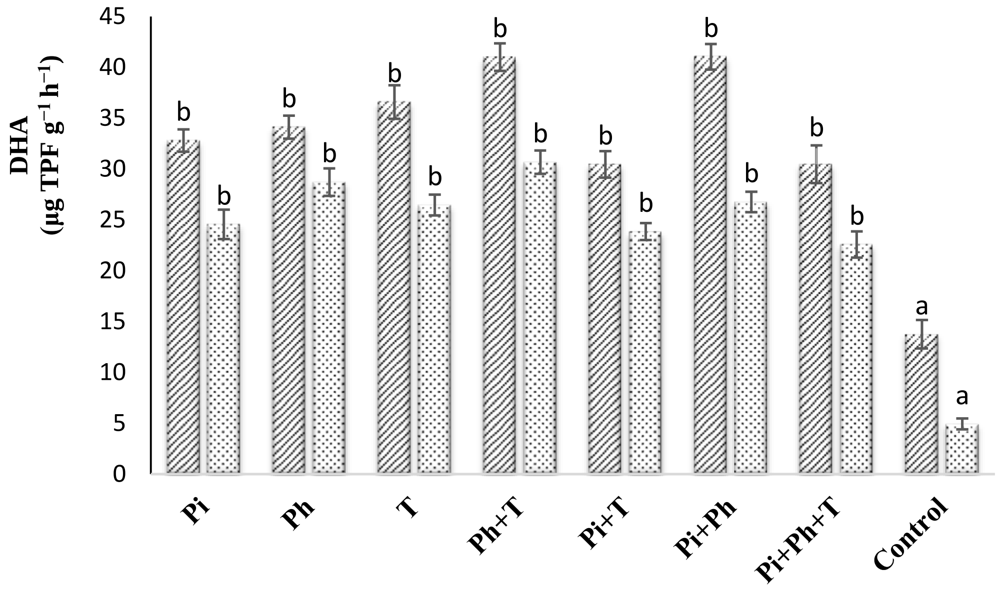


Fig. 5.1 Vertical difference in activity of DHA in two soil substrate layers within the different CWMs (mean ± SD). Various letters as superscripts signify the differences amongst DHA activity at $p < 0.05$

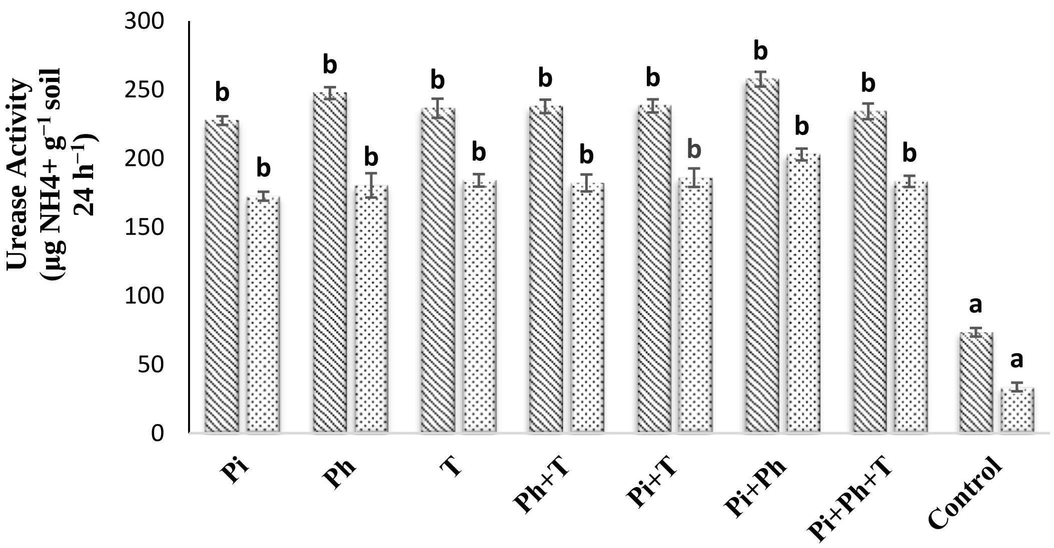


Fig. 5.2 Vertical difference in activity of Urease in two soil substrate layers within the different CWMs (mean ± SD). Various letters as superscripts signify the differences amongst urease activity at $p < 0.05$

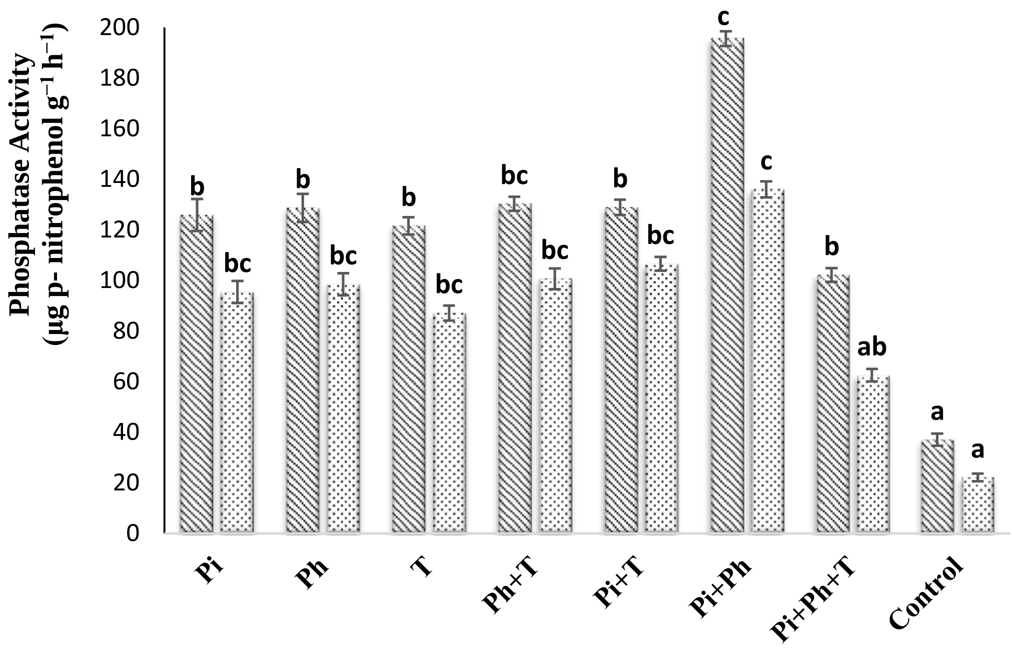


Fig. 5.3 Vertical difference in activity of Phosphatase in two soil substrate layers within the different CWMs (mean ± SD). Various letters as superscripts signify the differences amongst phosphatase activity at $p < 0.05$

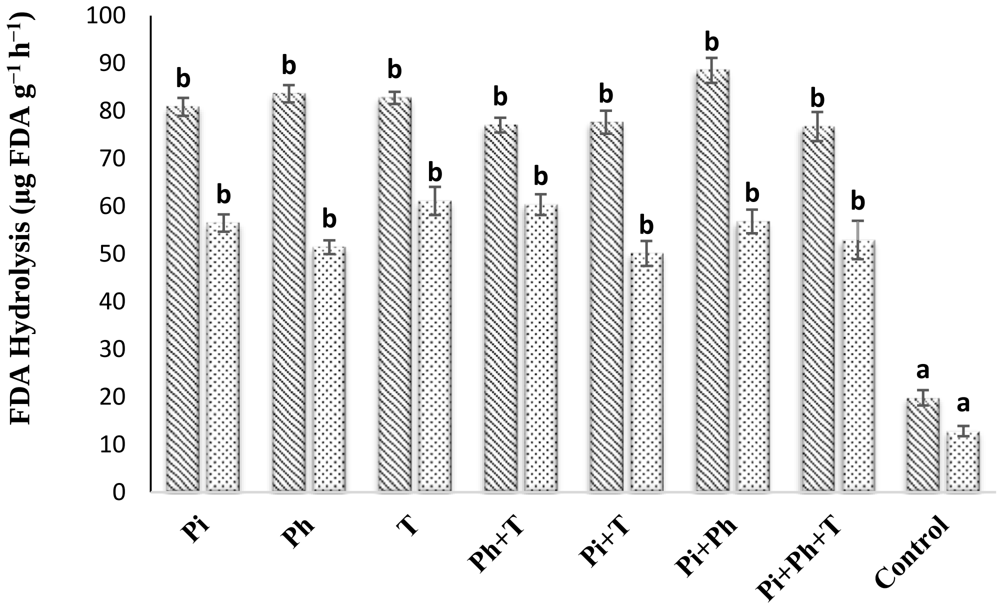


Fig. 5.4 Vertical difference in FDA hydrolysis in two soil substrate layers within the different CWMs (mean ± SD). Various letters as superscripts signify the differences amongst FDA hydrolysis at $p < 0.05$

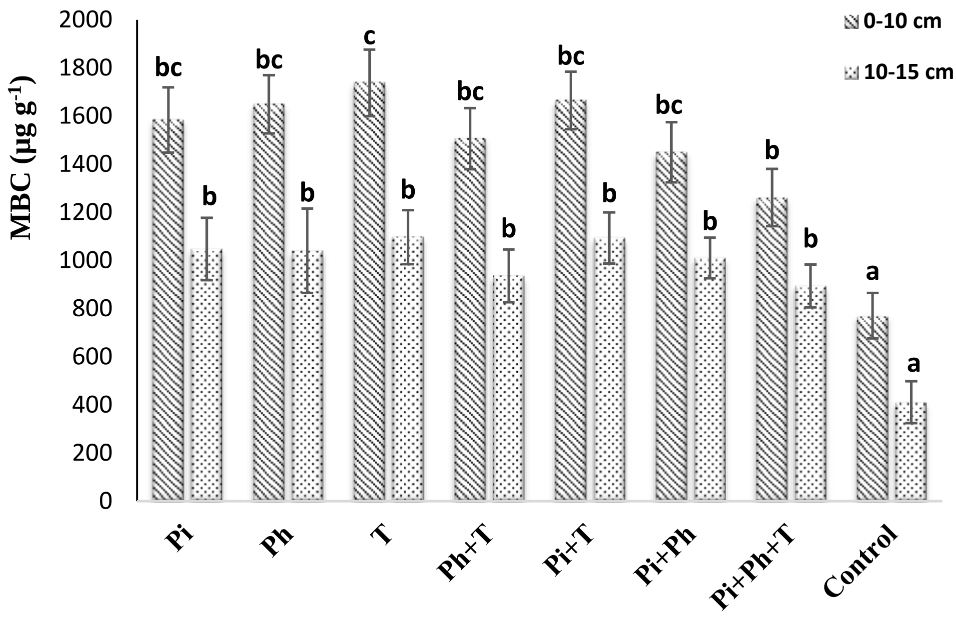


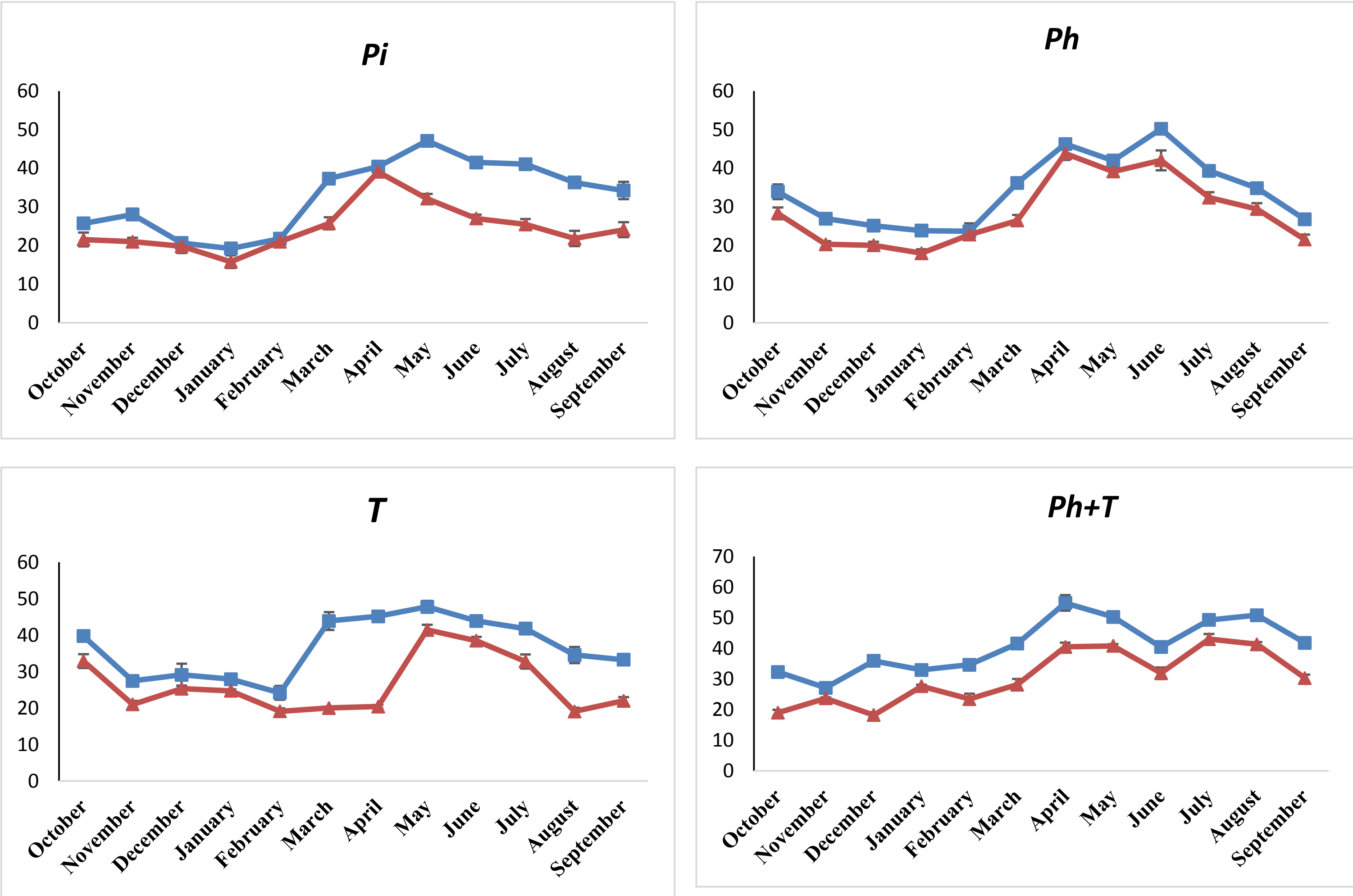
Fig. 5.5 Vertical difference in MBC in two soil substrate layers within the different CWMs (mean ± SD). Various letters as superscripts signify the differences amongst MBC at $p < 0.05$

It is well established that biomolecules have an important role in the conversion of nutrients via catalytic actions. It is also reported that the major portion of the organic matter within soil substrate is converted by the action of several enzymes through biochemical processes (Jackson et al., 2013). The assessment of activity of enzymes within soil substrate has been done earlier by several researchers to define microbial activity, microbial biomass and cycling of elements as a possible mechanism of the bioremediation process (Wang et al., 2007; Romani et al., 2012). Several studies dealing with various soil enzymes for numerous resolutions have been done previously. Urease, phosphatase and DHA are well-recognized enzymes known for their vital role in the alteration of organic matters and cycling of plant nutrients. It is established in numerous earlier studies that the activity of enzymes has an inverse relation with the depth of soil substrate, i.e., increase in depth may decrease the activity of several enzymes (Niemi et al., 2005; Kong et al., 2009; Kumar et al., 2020b). Similar results were also exhibited in this study, the uppermost layers exhibited significantly higher enzyme activities as compared to lower layers. The results were supported by the study carried out by Megha and Simpy (2014) in which they advised that more than half of the microorganism's biomass and conversion reactions take place in the upper layer up to 10 cm). This may be due to the reduction in microorganisms, moisture, organics and nutrients in lower layers. The variances may be also due to the various CWMs planted using emergent and free-floating macrophytes. Consequently, the top layer of CWM units having soil as a substrate material is crucial for the breakdown of contaminants.

5.3.2 Temporal variation in enzymes activity

The action of all selected enzymes such as DHA, phosphatase, urease, FDA, and MBC revealed significant differences depending upon time. The dissimilarity in the activity of all enzymes exhibited a nearly parallel pattern between all the wetland units. The maximum activity of DHA was recorded in June in the top layer of soil substrate for CWM unit Pi+Ph. However, for the deeper layer, the higher activity was revealed in April and July by CWM unit Ph and Ph+T respectively (Fig. 5.6). Various other wetland units also displayed the almost parallel activity of DHA. However, the control unit characterized the higher activity of DHA in June and October for both layers respectively. The highest activity of urease was recorded in March and April for deeper as well as in the top layer of the CWM units Ph and Pi+Ph respectively (Fig. 5.7). However, other CWM units also exhibited approximately equivalent activity of urease in, May, June and October. Both the top and deeper layers of the control unit exhibited the highest urease activity in May and June respectively. Phosphatase enzyme showed highest activity in May and October respectively for the upper and deeper layers within the CWM unit Pi+Ph (Fig. 5.8). Though, the control unit displayed maximum activity in April in both tops as well as the lower layers of soil substrate. Accordingly, the maximum FDA hydrolysis was observed in June for the top layer of CWM unit Pi+Ph (Fig. 5.9). However, for the deeper layer of soil substrate, there was a slight difference; with maximum also in June in CWM unit T throughout the experiment. The unplanted unit as control presented advanced FDA hydrolysis in May and October for both layers of soil substrate. MBC exhibited multi-peaks pattern for the top layer among various CWM units, however, the bottom layer of soil substrate showed advanced values in March and May in CWM unit T (Fig. 5.10). The control unit revealed maximum values of MBC in March for both layers of soil substrate.

Generally, the complete activity of microorganisms in natural environments provides an estimated turnover of organic matters due to the decomposer microbial populations that are accountable for about 90% of the energy transfer (Green et al., 2006). Sequestration of carbon in the natural ecosystem is identified as one of the important means to lessen climate change internationally (IPCC, 2003; Smith et al., 2005). Therefore, microorganisms’ biomass is measured as the prime component of the natural ecosystems responsible for nutrient cycling and transfer of energy. It is reported that the activity of various enzymes inside the soil substrate is accountable for enhancing water quality within CWM systems. It is also reported that these soil enzymes exhibited varied activity depending upon retention time and macrophytic species, having the lowest and highest peaks in various months of the year (Kang et al., 1998).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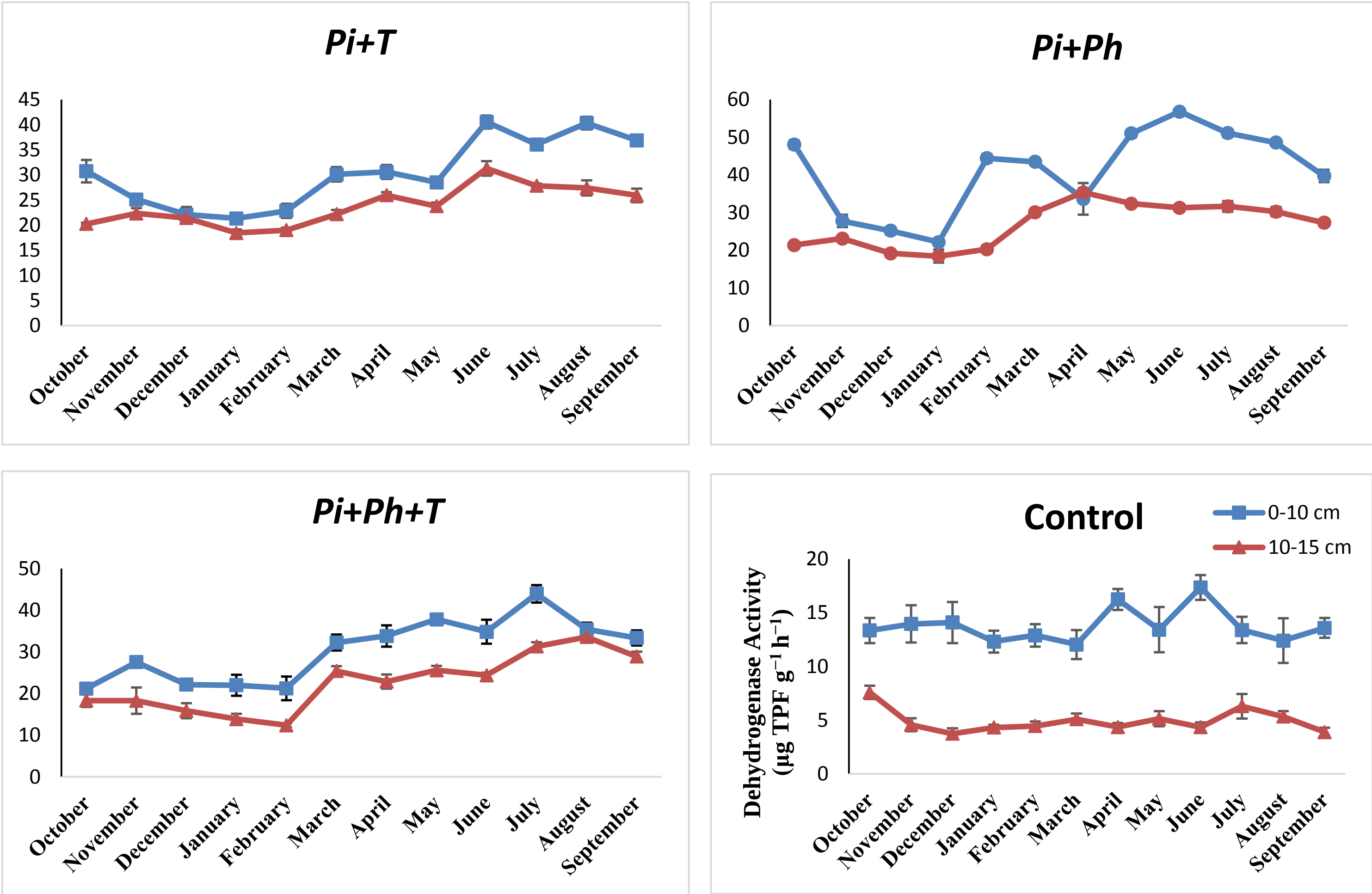
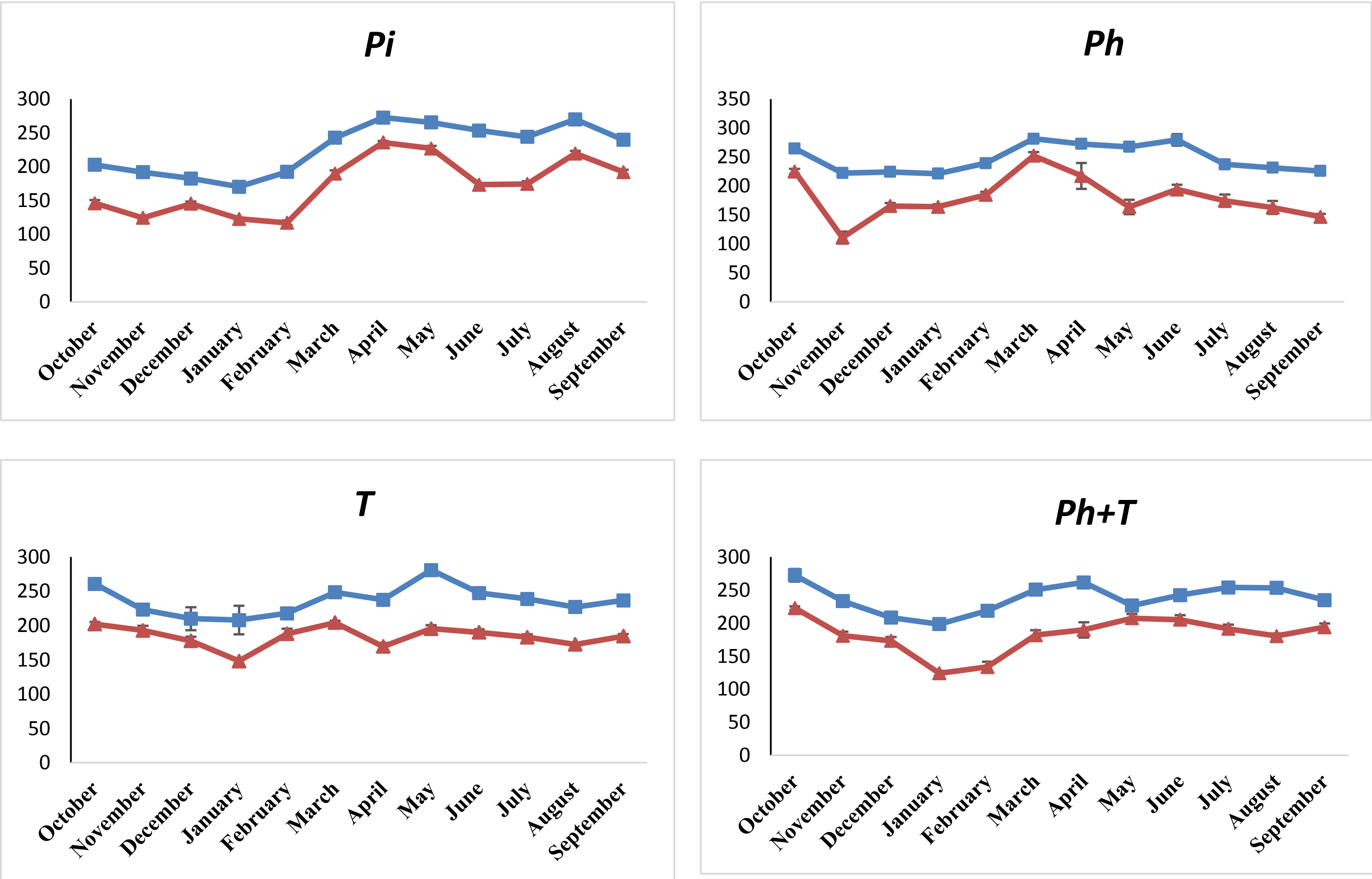


Fig. 5.6 Temporal variation in DHA activity at two soil layers of various CWM units (mean ± SD, n= 12)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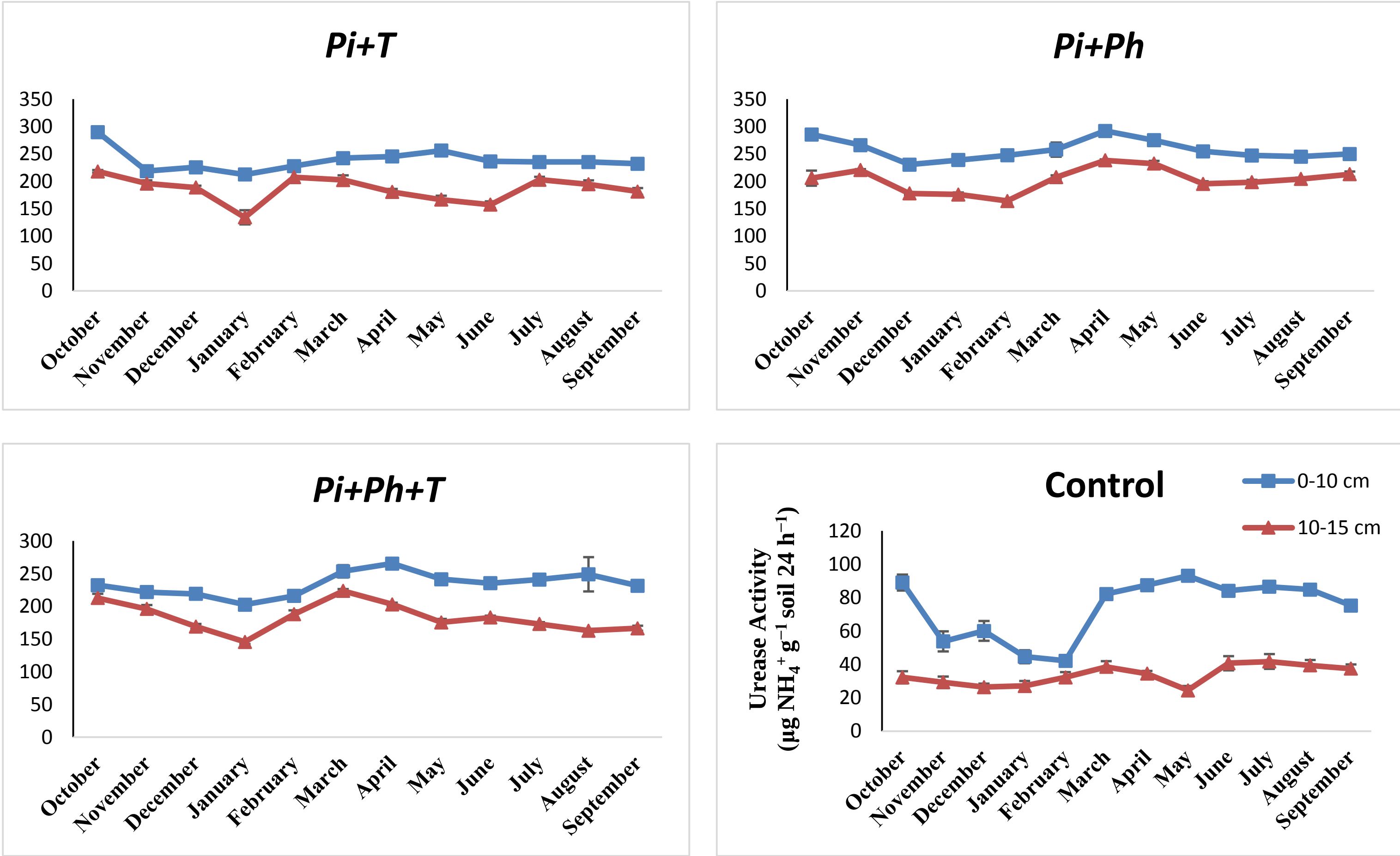
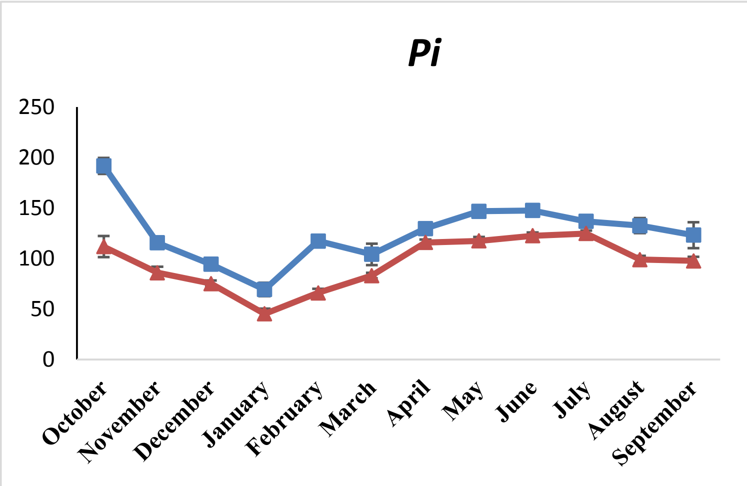


Fig. 5.7 Temporal variation in the activity of urease at two soil layers of different CWM units (mean ± SD, n= 12)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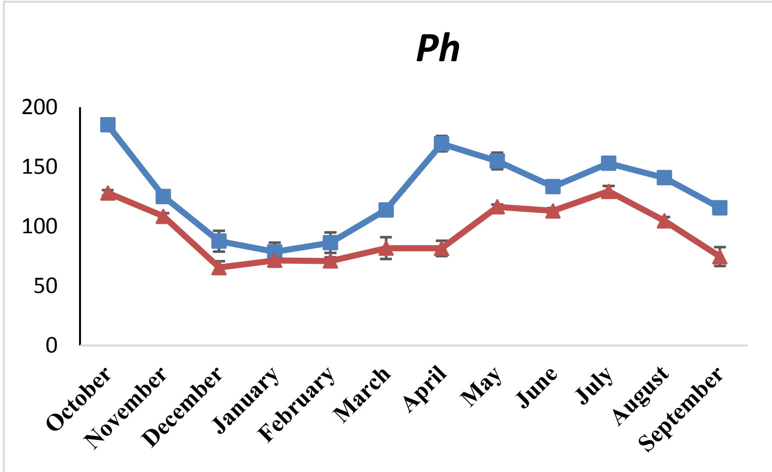
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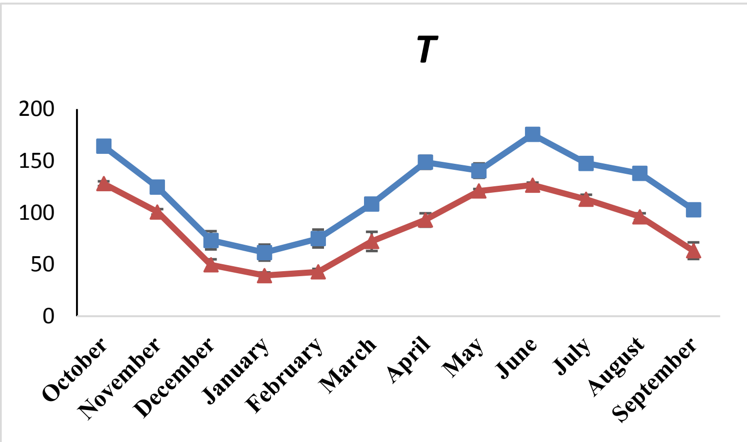
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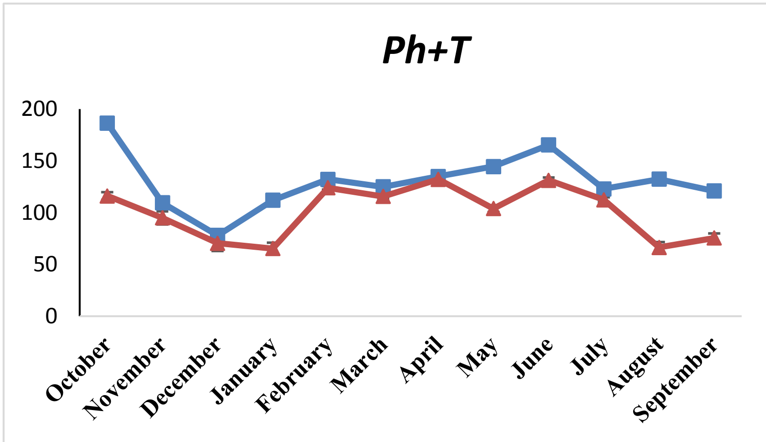
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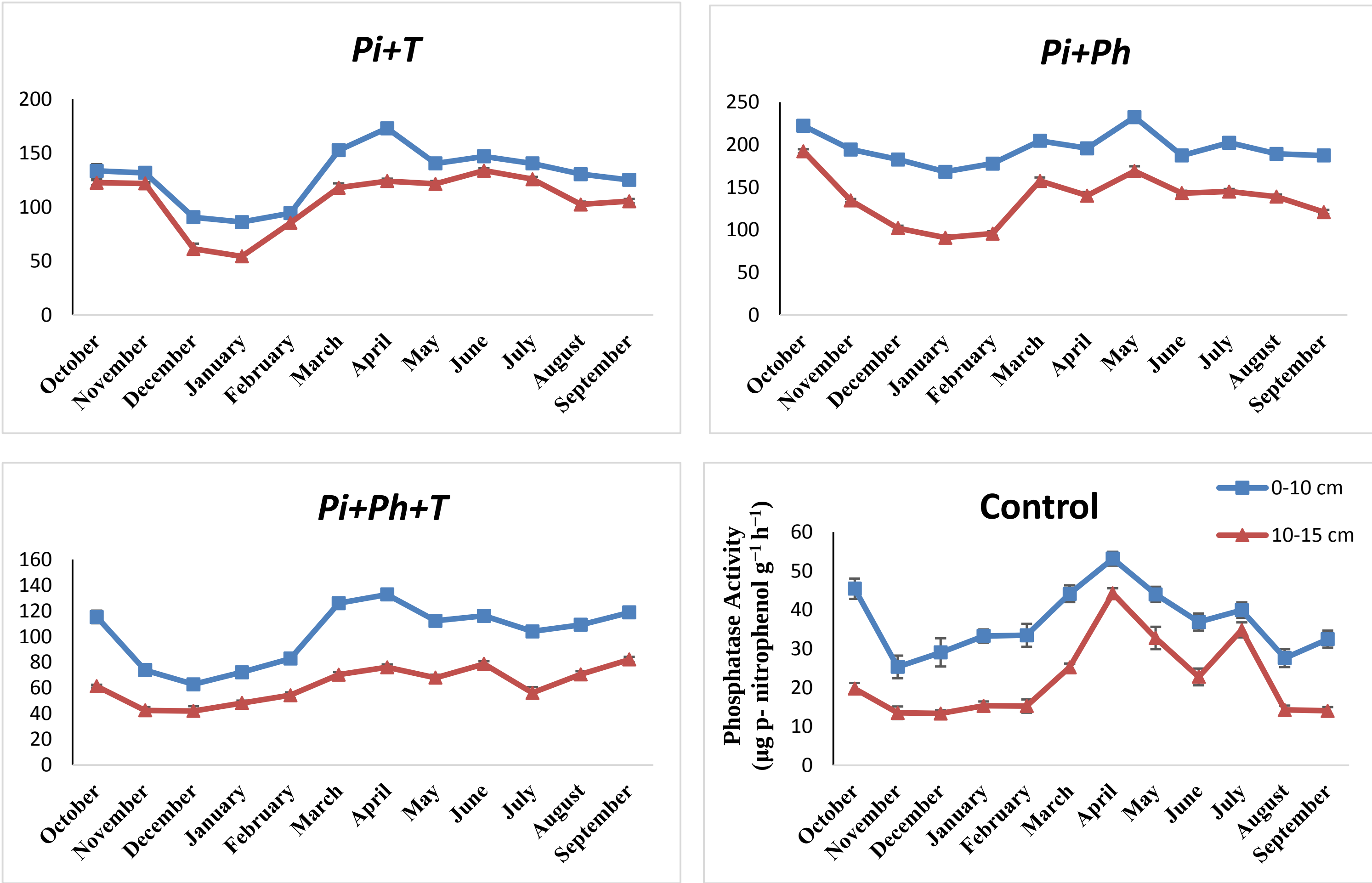
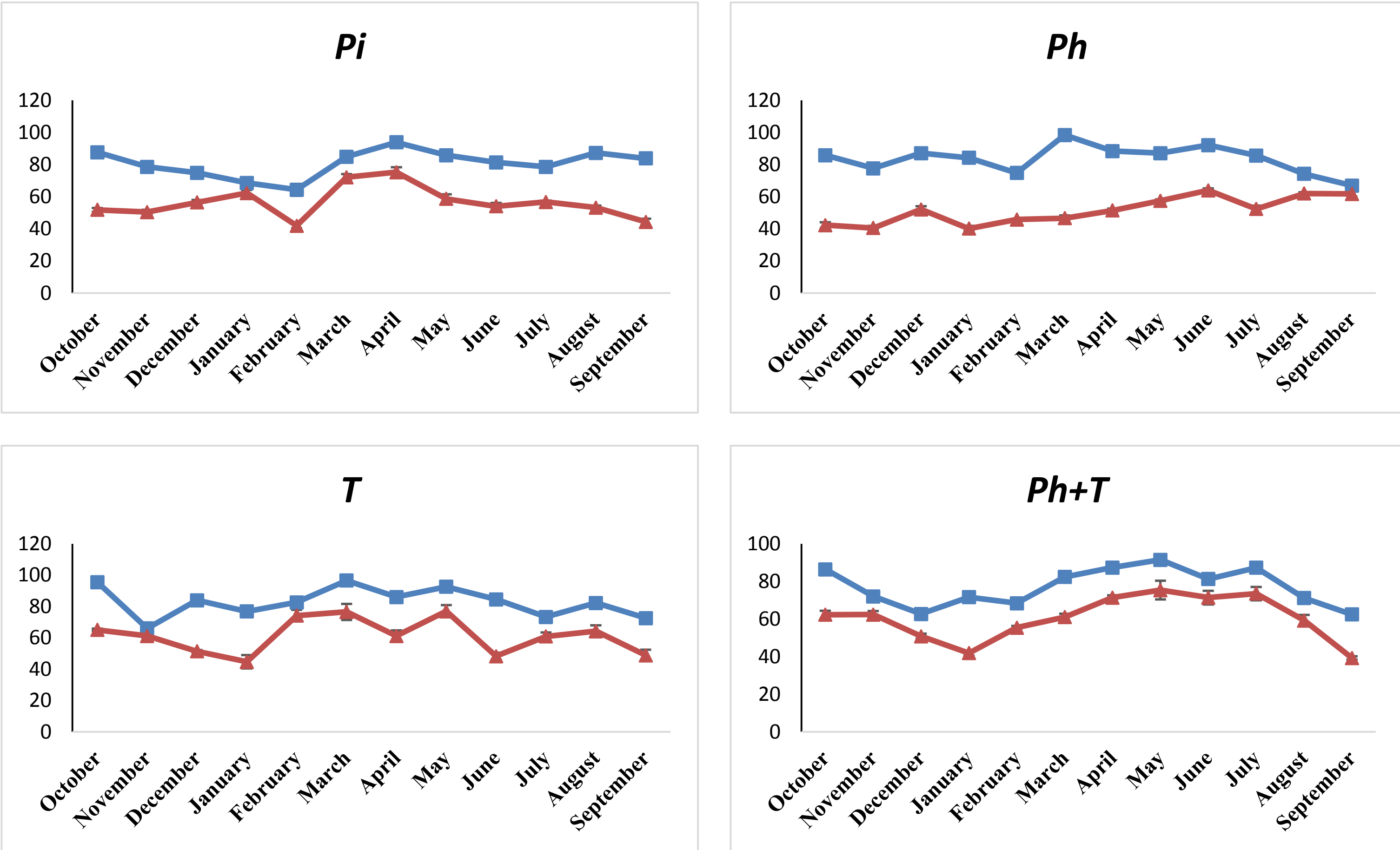


Fig. 5.8 Temporal variation in the activity of Phosphatase at two soil layers of different CWM units (mean ± SD, n= 12)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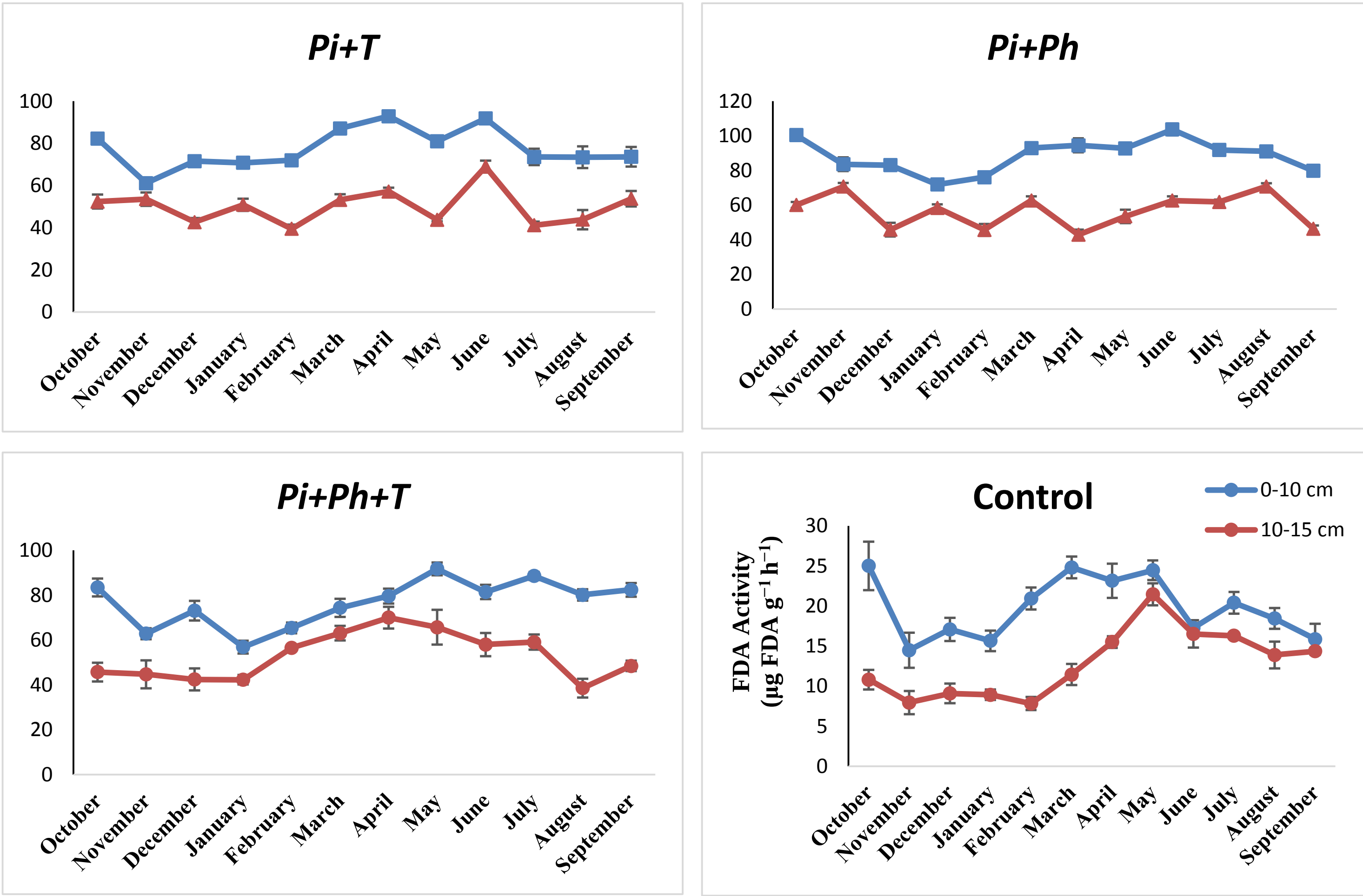
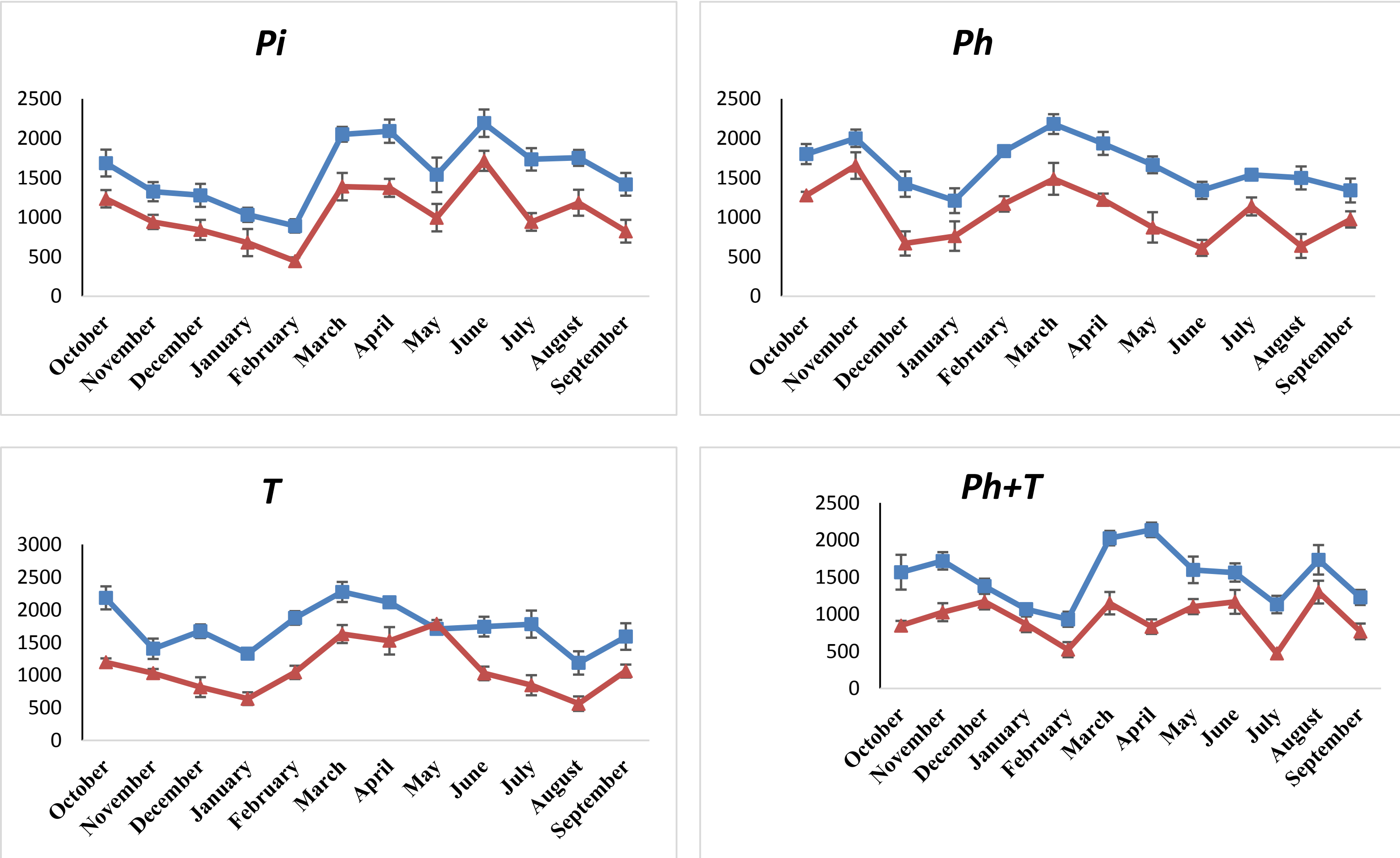


Fig. 5.9 Temporal variation in FDA hydrolysis at two soil layers of different CWM units (mean ± SD, n= 12)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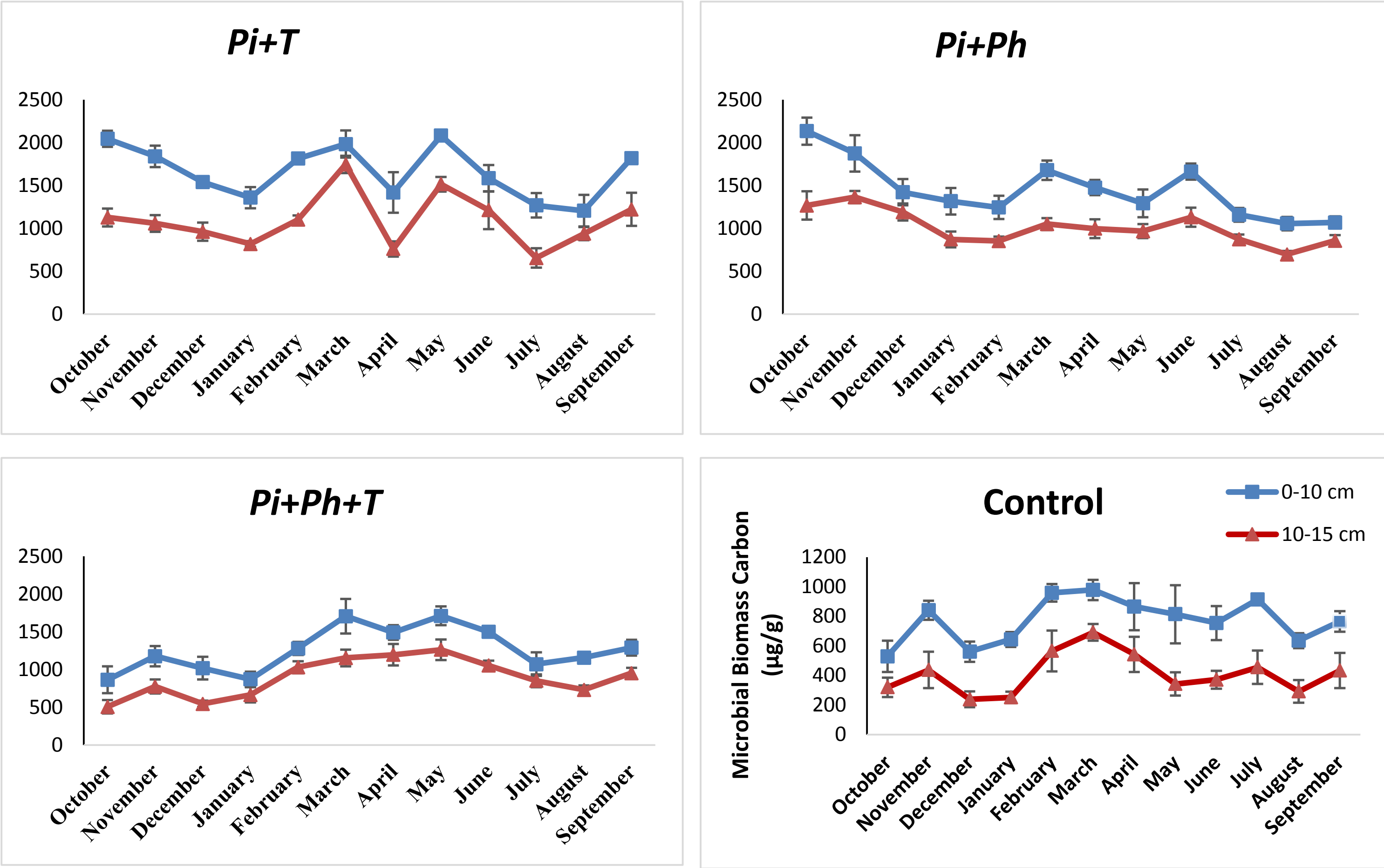

Fig. 5.10 Temporal variation in MBC at two soil layers of different CWM units (mean ± SD, n= 12)

5.3.3 Correlation among enzymes activity and contaminants removal

The results from the Pearson correlation coefficient at two significance levels ($p < 0.01$ and $p < 0.05$ respectively) indicate that there is a strong correlation between TP removal and phosphatase. Most of the CWM units exhibited a significantly positive correlation between phosphatase activity and TP removal at all three retention times and depths (Table 5.2). However, the removal of SRP showed a positive correlation with phosphatase activity in CWM units Pi, Ph and Pi+Ph and a significantly negative correlation in units Ph+T, Pi+T and Pi+Ph+T. Removal of NH_4^+-N and activity of urease exhibited a positive correlation in the majority of the CWMs together with significant correlation in Pi+Ph and Pi+Ph+T. The activity of urease at two soil depths and removal of NO_3^--N expressed a negative correlation excluding the CWM units Ph and Pi+T. The negative correlation between urease activity and NO_2^--N exclusion was also detected at 3 and 7 d retention times, however, positive for 14 d in the majority of CWM units. Accordingly, removal of BOD and activity of DHA also revealed a negative correlation among several CWM units except for Pi+T and Pi+Ph+T at 7 d retention time. Most of the CWMs exhibited a positive correlation between BOD elimination and FDA hydrolysis at 3 and 14 d retention times. However, CWM units Pi+Ph and Pi+Ph+T showed a significantly negative correlation. Exclusion of BOD was also significantly positively correlated with MBC in Pi, Pi+T and Pi+Ph units and negatively correlated with CWM unit T and Pi+Ph+T. Based on the macrophytes availability, phosphatase activity was found positively correlated with TP elimination in CWM units having *Phragmites karka* + *Typha latifolia* as compared to the other mixed as well as single planting units of three macrophytes.

Table 5.2 Correlation coefficients between contaminants removal and enzyme activities in different CWM units at two depths (mean, n =12)

Enzyme activities / Removal efficiencies	***Pi***		***Ph***		***T***		***T+Ph***		***T+Pi***		***Ph+Pi***		***T+Ph+Pi***		**Control**	
	0-10 cm	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**
Phosphatase-TP (3 d)	0.583	0.776**	0.737**	0.632*	0.212	0.362	-0.314	-0.455	0.004	-0.006	-0.341	-0.461	-0.201	-0.214	-0.612*	-0.361
Phosphatase-TP (7 d)	0.334	0.575*	0.029	0.309	0.765**	0.757**	0.688*	0.472	0.447	0.295	0.241	0.359	0.267	0.214	0.283	0.437
Phosphatase-TP (14 d)	0.211	0.011	0.657*	0.531	-0.223	-0.092	-0.055	0.040	-0.068	-0.258	-0.109	-0.282	-0.535	-0.523	-0.317	-0.282
Phosphatase-SRP (3 d)	0.432	0.262	0.306	0.313	-0.002	0.092	-0.809**	-0.615*	-0.315	-0.577*	0.006	-0.154	-0.672*	-0.616*	-0.259	-0.067
Phosphatase-SRP (7 d)	0.287	0.102	-0.219	-0.069	-0.259	-0.106	0.025	-0.513	-0.199	-0.110	0.495	0.501	-0.323	-0.343	0.624*	0.535
Phosphatase-SRP (14 d)	0.320	-0.034	-0.210	-0.272	-0.040	0.057	-0.007	0.001	0.031	0.072	0.087	0.209	-0.467	-0.467	-0.670*	-0.619*
Urease -NH_4^+-N (3 d)	-0.196	-0.049	-0.221	0.093	0.163	0.001	0.143	-0.252	0.231	0.254	0.112	-0.92	-0.005	-0.111	-0.397	-0.845**
Urease -NH_4^+-N (7 d)	-0.329	-0.384	0.202	0.023	0.182	0.484	0.396	0.327	0.009	0.336	0.504	0.414	0.736**	0.193	0.521	0.073
Urease -NH_4^+-N (14 d)	0.216	0.240	0.352	0.247	-0.011	0.123	0.287	0.471	0.363	-0.137	0.595*	0.744**	0.387	0.095	-0.400	-0.454
Urease -NO_2^--N (3 d)	-0.745**	-0.745**	-0.142	-0.336	-0.574	-0.108	0.148	0.071	-0.283	0.288	0.021	0.334	-0.272	-0.261	0.153	0.126
Urease -NO_2^--N (7 d)	-0.215	-0.243	-0.335	-0.157	0.209	-0.022	0.275	-0.036	0.074	-0.023	0.013	-0.162	0.310	0.366	-0.226	-0.437
Urease -NO_2^--N (14 d)	-0.436	-0.468	0.137	0.084	0.127	0.329	0.327	0.320	0.546	0.285	0.532	0.383	0.010	0.624*	-0.188	0.034
Urease -NO_3^--	-0.470	-0.436	0.220	0.116	0.076	0.095	-0.125	-0.076	0.128	0.011	0.183	-0.002	-0.100	0.046	-0.070	-0.419

Enzyme activities / Removal efficiencies	***Pi***		***Ph***		***T***		***T+Ph***		***T+Pi***		***Ph+Pi***		***T+Ph+Pi***		**Control**	
	0-10 cm	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**	**0-10 cm**	**10-15 cm**
N (3 d)																
Urease -NO_3^--N (7 d)	-0.360	-0.337	-0.048	-0.171	-0.035	-0.186	0.045	-0.146	0.083	0.227	0.313	0.094	-0.128	0.233	0.269	0.311
Urease -NO_3^--N (14 d)	-0.349	-0.366	0.061	0.081	-0.207	0.010	-0.086	-0.148	0.021	0.181	-0.002	-0.247	-0.398	-0.010	-0.160	-0.195
DHA- BOD (3 d)	-0.586	-0.402	-0.543	-0.433	-0.420	-0.263	-0.327	-0.355	-0.429	-0.520	-0.523	-0.240	-0.374	-0.135	0.050	-0.125
DHA- BOD (7 d)	0.006	-0.041	-0.058	-0.214	-0.227	-0.179	-0.216	-0.240	0.402	0.384	0.034	-0.080	0.035	0.280	-0.325	-0.101
DHA- BOD (14 d)	-0.125	-0.067	-0.460	-0.471	-0.196	-0.078	-0.289	-0.368	-0.173	-0.162	-0.029	0.126	-0.359	-0.316	-0.407	-0.174
MBC-BOD (3 d)	-0.550	-0.591*	0.038	-0.039	0.041	-0.196	0.241	0.507	-0.100	-0.171	0.167	0.415	-0.493	-0.578*	-0.321	-0.365
MBC- BOD (7 d)	0.468	0.419	-0.010	0.220	0.097	-0.592*	-0.128	-0.221	-0.307	-0.387	0.391	0.279	-0.631*	-0.700*	-0.184	-0.020
MBC- BOD (14 d)	-0.162	-0.352	0.216	0.477	0.224	0.024	0.108	0.081	-0.107	-0.398	-0.121	-0.17	-0.507	-0.589*	-0.240	-0.142
FDA- BOD (3 d)	-0.378	-0.125	-0.501	-0.116	-0.041	0.011	-0.421	-0.516	-0.515	-0.131	-0.159	-0.648*	-0.257	-0.620*	-0.627*	-0.076
FDA- BOD (7 d)	0.205	0.020	0.014	-0.221	-0.165	-0.389	-0.072	0.044	0.198	0.396	0.295	0.330	0.088	-0.579*	-0.319	-0.617*
FDA- BOD (14 d)	0.044	0.138	-0.163	-0.569	0.392	0.339	0.044	-0.047	-0.237	-0.175	0.071	-0.039	-0.119	-0.327	-0.067	-0.539

** Correlation significant at $p < 0.01$ level

* Correlation significant at $p < 0.05$ level

The correlation results between the activity of enzymes and contaminant removal effectiveness varied greatly concerning contaminants, enzymes, CWM unit depths and retention time. The exclusion efficacy of TP was positively correlated with the activity of phosphatase in nearly all the CWMs. However, the correlation between SRP removal and phosphatase activity was greatly affected by available macrophytes. The abundance and activity of phosphatase enzyme in the soil represent the presence of available phosphorus, responsible for the conversion of organic into inorganic and labile forms via hydrolysis. It is considered a vital oxidoreductase enzyme present in soil substrate that poses a critical role in the cycling of phosphorus. Urease is commonly measured as a hydrolase enzyme liable for hydrolytic modification of the urea into NH_3 and CO_2. Measurement of the activity of the urease enzyme is crucial to comprehend nitrogen mineralization. However, the hydrolysis of urea through non-enzymatic processes mainly occurs at a low rate (approx. 0 – 2%) as discussed earlier (Thoren, 2007). Removal of NO_2^--N and NO_3^--N exhibited a positive and negative correlation with urease enzyme depending upon macrophyte combination and retention time. It is supposed that the major nitrogen form existing in DW as NH_4^+ restricts the process of nitrification because of lower DO. Consequently, a moderate association was detected among the above-explained pairs. DHA plays a crucial role in the early oxidation of organic matter via transferring electrons or H_2 to acceptors from soil substrate (Phale et al., 2019). It is also measured as a possible indicator of microbiological action inside the substrate materials. The activity of DHA and BOD removal also revealed both negative and positive correlations reliant upon macrophytes, soil depth and retention time. Analysis of the FDA hydrolysis and MBC within soil substrate samples gives insight into the activity of microorganism populations and also provides estimates of comprehensive microbial activities. It is discussed earlier that the activity of several extracellular enzymes within substrates of wetlands directly takes part in the purification mechanism of wastewater (Kong et al., 2009). The removal efficacies of all selected water quality parameters depending upon different CWM units and retention times throughout the year have been provided in table 5.3- 5.8.

Table 5.3 Percent removal of BOD throughout the year with respect to different treatment units and retention time (mean± SD)

Months	**Treatment units**							
3 Days	**Pi**	**Ph**	**T**	**Ph+T**	**Pi+T**	**Pi+Ph**	**Pi+Ph+T**	**Control**
October	53.21±0.35	54.47±0.30	51.55±0.40	52.38±0.50	56.52±0.31	57.14±0.31	59.74±0.25	16.69±0.20
November	45.63±0.50	61.76±0.53	53.23±0.25	62.50±0.36	61.48±1.00	56.91±0.50	64.44±0.15	24.92±0.50
December	65.12±0.50	63.74±0.35	70.10±0.60	60.22±0.25	65.22±0.42	69.60±0.31	55.05±0.25	16.21±0.50
January	52.08±1.50	48.53±1.23	46.10±1.10	59.53±0.60	60.00±0.42	53.33±0.40	52.27±0.81	19.05±2.56
February	54.17±0.76	58.02±0.35	56.02±0.95	43.09±0.76	53.09±0.61	55.78±0.31	48.63±0.25	11.38±1.44
March	49.61±0.50	42.77±0.46	53.83±0.40	54.10±0.42	57.46±0.40	58.56±0.12	48.82±0.36	15.09±0.64
April	47.16±0.31	52.08±0.75	48.12±0.61	50.73±0.53	53.17±0.76	61.82±0.53	51.76±0.51	16.92±2
May	50.00±0.50	48.40±0.58	48.99±0.60	55.21±0.58	41.88±0.50	57.98±0.50	49.90±0.50	15.91±1.00
June	40.48±1.53	45.12±1	46.07±2	40.28±1.53	44.29±1	52.31±0.58	41.05±0.58	17.95±1
July	51.74±1.5	31.88±0.85	53.68±1.3	37.04±0.8	47.42±0.5	48.98±1.5	48.00±1.8	15.71±0.8
August	48.86±1	58.33±0.5	51.39±1.5	55.88±1	57.58±0.45	50.41±2.1	57.45±1.5	22.20±2.3
September	50.86±1	55.33±1	53.39±0.9	56.88±1.4	58.58±0.5	60.41±1.8	55.45±1.4	20.20±2
7 Days								
October	77.54±0.15	89.46±0.41	83.37±0.15	85.60±0.21	84.23±0.25	83.20±0.06	86.80±0.15	27.48±0.36
November	70.00±0.50	81.67±0.58	75.00±0.40	76.67±0.25	73.33±0.50	80.00±0.44	83.33±0.50	26.67±0.51
December	72.44±0.50	77.61±0.50	78.85±1.32	80.77±1	80.34±2.52	78.63±1.53	79.27±1.76	38.03±1.53
January	70.00±1	77.33±1.03	78.13±0.50	73.33±1.53	70.83±1.53	77.50±1	68.75±1	21.25±1
February	70.21±1	71.28±1	73.48±0.90	75.64±0.85	66.74±0.64	75.78±1.57	66.31±0.58	30.85±2
March	77.72±0.90	79.08±1.73	76.54±0.29	73.25±1.53	72.37±1	80.53±0.72	70.18±1.53	26.75±1.15
April	74.29±1	74.76±1.31	73.02±0.58	76.03±1.04	75.94±0.65	79.05±1	69.27±0.31	17.46±0.58
May	60.66±1	59.59±0.70	60.46±0.75	59.84±1	59.97±0.76	61.75±0.58	57.38±1	13.93±2

Months	**Treatment units**							
June	78.57±1	79.37±0.58	76.59±1.53	83.33±1	84.13±1.53	86.11±1.53	74.21±1.53	19.05±1
July	77.37±1.5	88.17±0.95	83.71±1.6	86.11±0.75	83.37±1.8	83.20±2.3	87.14±1.2	27.82±2.4
August	71.67±1.2	81.67±1	76.67±0.75	78.00±1.3	72.83±2	78.67±2.7	85.00±1.5	25.50±2.5
September	78.87±2.31	77.03±3.51	73.75±1.53	73.75±3.21	76.12±1.15	76.38±3	78.74±1.73	32.81±0.58
14 Days								
October	88.92±1	95.71±0.54	91.94±1.2	94.00±1.5	92.69±1.5	92.34±1.5	94.27±2	39.59±1.5
November	84.17±1.2	92.33±0.65	88.28±1.2	90.67±1.5	90.00±0.92	90.78±1.5	93.56±2.5	44.1±12
December	90.38±1.3	91.88±2	93.68±1.5	92.35±1.2	93.16±1.6	93.50±1.5	90.68±3	48.08±2.6
January	85.63±1.2	88.33±1.2	88.21±1.4	89.21±0.75	88.33±1.1	89.50±1.4	85.08±2.6	36.25±3.5
February	86.35±0.5	87.94±1	88.33±1	86.13±0.5	84.40±1	89.29±1.6	82.70±1.5	38.72±0.28
March	88.77±0.8	88.03±0.62	89.17±1	87.72±0.5	88.25±1.26	91.93±1.8	84.74±1.8	37.81±1.6
April	86.41±2	87.90±0.42	86.00±1	88.19±0.56	88.73±0.63	92.00±1.8	85.17±2	31.43±1.8
May	87.30±1.5	86.34±1	86.94±1.5	88.25±1.3	84.84±0.85	89.78±1.2	85.63±2.2	39.34±1.5
June	80.16±1.4	82.14±2.5	80.95±1.4	82.94±2	84.52±1	87.70±0.8	77.78±0.85	23.81±2.3
July	90.87±0.9	92.86±1.5	84.95±0.95	88.94±2.6	94.52±1.5	97.70±1.3	87.78±0.76	26.81±2
August	86.30±0.52	87.34±0.5	85.94±0.8	89.25±3	84.84±1.8	90.78±1.5	83.63±1	31.34±2
September	87.30±0.86	89.34±1.4	75.94±1	85.25±1.8	88.84±2	91.78±1	81.63±2.3	37.34±2.5

Table 5.4 Percent removal of TP throughout the year with respect to different treatment units and retention time (mean± SD)

Months	**Treatment units**							
3 Days	**Pi**	**Ph**	**T**	**Ph+T**	**Pi+T**	**Pi+Ph**	**Pi+Ph+T**	**Control**
October	60.29±0.16	49.07±0.22	50.85±0.33	46.93±0.33	57.29±0.45	50.00±0.08	53.95±0.36	19.35±0.61
November	46.33±0.34	52.00±0.32	60.00±0.20	54.48±0.08	36.86±0.32	75.25±0.06	71.88±0.04	32.37±0.50
December	56.08±1.15	52.73±1.35	59.85±0.80	56.43±1	61.06±0.95	74.85±0.65	64.52±0.75	43.56±2.15
January	47.62±2.50	45.61±2.80	50.69±2.40	44.45±2.04	49.04±2.03	53.73±1.21	44.17±2	23.46±5.65
February	42.31±0.16	41.38±0.32	43.25±0.05	51.98±0.32	44.98±0.32	56.02±0.26	47.76±0.43	16.09±0.40
March	45.13±0.40	48.84±0.40	45.95±0.36	43.14±0.40	43.26±0.35	46.37±0.14	41.93±0.10	15.96±0.52
April	61.09±1.5	64.38±2.5	51.60±1.4	37.50±2.32	52.38±1.52	48.75±0.56	58.16±0.85	15.61±1.62
May	54.72±1.25	50.68±1.5	64.01±1.56	54.27±2.45	69.27±1.5	50.23±0.45	55.56±0.84	21.95±1.5
June	60.89±1.54	63.82±1.4	53.04±1.23	47.50±2.42	45.38±1.42	49.47±0.95	50.16±1.5	19.61±2.05
July	62.48±1.5	63.30±1.5	60.42±1.02	68.12±2.74	61.88±1.26	57.27±0.78	64.55±1.52	32.55±3.25
August	54.29±1.56	50.00±1.6	53.33±1.45	67.21±1.56	38.40±0.95	54.36±0.82	49.23±1.47	25.83±1.75
September	49.64±2.26	41.94±1.8	58.57±1.54	60.00±1.78	44.44±0.85	72.73±0.64	67.42±1.85	27.27±1.56
7 Days								
October	58.03±0.20	50.45±1.16	73.18±0.10	72.88±0.21	69.85±0.20	67.42±0.05	62.27±0.15	29.39±0.49
November	53.65±0.15	58.33±0.25	59.62±0.51	62.82±0.35	55.13±0.25	48.72±0.15	55.77±0.35	24.36±0.15
December	54.15±2.40	48.88±2.80	59.11±2.05	56.63±2.35	64.99±1.95	47.03±2.90	61.59±2.05	30.30±3.90
January	59.54±4	56.07±4.83	58.25±4.50	64.68±3.35	64.84±3.45	72.86±3	64.74±3.49	29.51±7.30
February	51.08±0.50	52.82±0.25	55.86±0.68	56.08±0.31	48.47±0.66	58.47±0.35	45.64±0.15	18.90±0.40
March	50.43±0.76	44.75±0.71	64.43±0.33	64.83±0.35	67.57±0.88	67.68±0.24	57.39±0.35	18.13±0.25
April	61.06±1.5	43.64±1.5	71.67±2.3	72.12±1.26	71.36±1.2	66.74±2.5	63.79±1.5	28.64±1.8
May	59.85±2	63.03±1.4	72.42±2.5	75.15±2.36	72.88±1.5	67.27±2.1	64.55±1.5	35.45±2.56

Months	**Treatment units**							
June	61.06±2.3	43.64±1.6	71.67±2.5	72.12±2.85	71.36±1.5	66.74±2.6	63.79±2.5	28.64±2.31
July	62.48±2	63.30±15	60.42±3.6	68.12±0.95	61.88±1.6	77.27±1.5	64.55±1.5	32.55±1.56
August	55.13±2.5	61.54±1.5	61.54±2.5	60.90±0.85	51.92±1.5	70.00±1.8	58.33±1.8	23.08±1.52
September	55.45±1.5	60.26±1	55.13±1.5	65.38±1.2	53.85±2.5	50.64±1.8	60.26±1.8	27.44±2.45
14 Days								
October	83.33±1.2	82.20±1.5	86.82±1.5	85.61±1.5	87.12±1.5	83.71±2.45	82.63±2.12	43.06±1.54
November	77.39±1.25	79.23±1.52	82.44±1.5	86.71±1.25	72.14±1.8	87.01±2.6	87.26±2.14	45.13±2.14
December	79.86±1.53	75.84±1.45	83.58±2.5	81.10±1.5	86.37±2.3	86.68±1.58	86.37±1.5	60.66±2.18
January	78.81±1.45	76.11±2.62	79.42±2	80.38±1.62	82.08±1.45	87.44±1.85	80.31±1.45	46.05±1.85
February	71.78±1.45	72.34±2	74.95±1.4	78.91±2.15	71.65±2.54	81.74±1.45	71.60±1.62	31.94±1.4
March	72.80±2.36	71.73±1.5	80.77±1.2	80.00±2.18	81.60±2	82.67±1.62	75.25±3.52	31.20±1.6
April	75.81±2.45	78.11±1	76.42±2	79.38±1.85	80.08±2.5	83.44±1.45	72.31±2.48	41.05±1.25
May	77.81±2.85	79.11±1.34	77.42±0.9	75.38±1.85	82.08±1.8	84.44±2.65	75.31±2.78	38.50±2.54
June	73.78±2.78	75.34±1.45	70.95±0.85	77.91±4.36	74.65±1.95	80.74±2.45	70.03±4.25	31.94±3.45
July	75.78±2.48	77.34±1.52	74.95±0.78	81.91±2.75	74.65±1.54	85.74±2.47	66.52±2.15	36.94±2.12
August	71.78±2.45	77.81±1.5	70.95±0.45	75.91±2	76.65±1.62	82.74±2	76.52±1.5	38.94±2.78
September	77.78±3.54	79.34±2.5	75.95±2.36	80.08±2.68	72.65±1.64	83.74±2.45	78.52±1.67	37.94±1.62

Table 5.5 Percent removal of SRP throughout the year with respect to different treatment units and retention time (mean± SD)

Months	**Treatment units**							
3 Days	**Pi**	**Ph**	**T**	**Ph+T**	**Pi+T**	**Pi+Ph**	**Pi+Ph+T**	**Control**
October	58.02±0.26	66.38±0.20	41.87±0.23	40.91±0.25	46.67±0.26	52.90±0.10	46.58±0.25	12.20±0.31
November	53.33±0.17	57.03±0.15	63.43±0.31	64.87±0.21	43.91±0.12	75.33±0.31	68.32±0.23	20.93±0.40
December	49.07±0.010	64.29±0.01	50.98±0.01	62.44±0.01	66.99±0.01	65.87±0.01	60.93±0.10	19.82±0.97
January	50.83±0.25	45.94±0.75	48.95±0.65	59.53±0.5	55.26±0.55	57.33±0.35	50.67±0.36	20.48±0.12
February	48.11±0.2	50.82±0.17	44.25±0.24	53.98±0.45	46.98±0.25	54.94±0.28	48.76±0.35	26.09±0.5
March	53.61±0.14	52.77±0.15	55.32±0.45	50.10±0.35	53.46±0.65	58.86±0.36	44.82±0.45	25.90±0.12
April	48.16±0.25	51.75±0.85	42.12±0.85	47.85±0.25	52.17±0.58	51.18±0.55	46.56±0.36	26.92±1.25
May	57.05±0.34	50.75±1	48.76±0.26	52.28±0.25	55.66±0.258	62.18±0.68	54.76±0.52	26.92±2.54
June	53.72±0.35	54.79±1.2	54.11±0.48	48.68±0.35	49.74±0.54	55.31±0.55	51.56±0.83	20.95±1.5
July	51.74±0.52	51.41±1.5	53.68±0.55	47.37±0.55	47.42±0.58	48.98±0.68	45.36±0.34	15.71±1.8
August	48.86±0.36	56.33±0.26	51.39±0.45	53.88±0.57	57.58±0.35	50.41±0.28	51.45±0.15	22.20±1.5
September	40.64±0.5	45.13±0.25	50.89±0.35	52.24±0.65	48.58±1	55.13±0.25	45.45±0.35	20.20±2.1
7 Days								
October	59.44±0.35	52.73±0.21	74.61±0.26	72.76±0.30	67.49±0.15	71.52±0.17	59.24±0.06	26.42±0.17
November	50.93±0.21	61.83±0.12	59.80±0.13	66.60±0.23	54.83±0.26	49.47±0.09	52.33±0.36	17.23±0.20
December	53.17±0.15	57.19±0.01	68.81±0.05	69.88±0.12	68.50±0.18	48.93±0.22	67.13±0.12	25.08±0.15
January	58.27±0.12	55.35±0.75	64.61±0.48	62.76±0.96	57.49±1	61.70±0.15	56.24±0.85	28.42±0.41
February	62.67±0.56	69.88±1.2	65.49±0.25	60.85±0.85	64.63±1	68.61±0.75	58.43±0.42	33.32±0.25
March	58.45±0.45	62.78±0.64	60.00±0.45	58.42±0.45	57.60±0.52	66.70±0.26	57.33±0.36	38.42±0.25
April	52.08±1	58.53±0.85	46.10±0.43	59.53±1	60.00±1.2	63.33±0.54	52.27±0.45	39.05±1.2
May	59.61±0.26	62.77±0.61	63.83±0.26	64.10±2.2	67.46±0.64	71.56±0.25	58.82±0.12	35.09±1

Months	**Treatment units**							
June	60.66±0.48	59.59±0.43	60.46±1.5	59.84±1.75	59.97±0.75	61.75±0.25	57.38±0.35	33.34±1.5
July	60.48±0.48	65.12±0.22	56.07±1	60.28±0.64	64.29±0.62	72.31±0.42	64.05±0.56	27.95±2
August	62.48±0356	64.30±0.15	60.42±1.2	68.12±0.48	61.88±0.26	67.27±0.41	60.55±0.45	32.55±1.82
September	58.86±0.85	63.33±0.36	61.39±0.54	65.24±0.94	57.58±0.55	60.41±0.36	54.45±0.85	32.20±1.45
14 Days								
October	84.00±0.25	82.04±0.54	86.62±0.52	86.65±0.74	83.69±0.54	86.38±0.62	80.98±1.5	38.84±0.56
November	77.10±0.35	83.60±0.36	85.30±1	88.27±0.52	74.67±0.5	87.53±0.36	84.90±1.2	42.83±0.45
December	76.15±0.36	84.71±0.45	84.71±1.2	88.69±0.45	89.60±0.6	82.57±0.64	87.16±1.6	40.52±0.25
January	75.26±0.52	77.33±0.25	73.13±1.5	72.33±0.26	68.33±0.55	79.50±0.52	65.75±0.58	32.25±1.2
February	73.78±0.45	75.42±0.62	77.95±0.25	78.85±0.95	70.65±0.6	83.74±0.62	74.03±0.85	35.42±1.25
March	75.93±0.25	80.79±0.5	76.54±0.56	72.25±0.75	73.84±0.74	82.63±.85	68.18±0.75	22.75±1.5
April	73.86±0.85	76.19±0.55	70.16±0.58	74.32±0.24	75.37±0.58	80.48±0.36	67.27±0.65	21.46±0.36
May	70.27±0.85	75.00±0.5	71.86±0.53	77.21±0.59	78.48±0.95	79.78±0.45	77.90±0.5	35.91±0.52
June	78.57±0.026	79.37±0.85	76.59±0.95	80.33±0.95	82.13±0.69	86.11±0.28	74.21±0.52	39.05±0.95
July	71.67±0.5	61.27±0.45	73.17±0.65	75.85±0.45	72.83±0.85	77.13±0.62	65.45±0.85	33.85±0.45
August	71.67±0.15	71.67±0.25	76.67±0.36	78.00±0.85	72.83±0.42	78.67±0.85	75.25±0.42	35.50±0.62
September	68.71±0.82	71.34±0.36	73.75±0.25	70.53±0.28	72.55±0.61	76.38±1.5	68.74±0.62	34.81±0.45

Table 5.6 Percent removal of NH_4^+-N removal efficiencies throughout the year with respect to different treatment units and time (mean± SD)

Months	**Treatment units**							
3 Days	**Pi**	**Ph**	**T**	**Ph+T**	**Pi+T**	**Pi+Ph**	**Pi+Ph+T**	**Control**
October	78.03±0.56	70.55±2.85	73.18±2.36	71.79±0.15	70.48±0.85	74.24±0.45	68.73±0.87	19.39±1.5
November	68.54±0.42	70.33±2.58	70.62±1.2	71.82±0.85	69.28±0.25	69.72±0.12	67.69±0.58	18.59±1.5
December	69.51±0.78	68.48±3.57	69.11±1.52	68.63±.24	67.99±1	67.26±0.65	71.59±0.78	20.30±1.2
January	69.38±1.25	67.69±1.63	68.25±1.45	69.76±0.24	70.36±1.5	71.64±0.05	64.74±0.86	21.12±1.8
February	71.76±1.5	66.16±1.2	79.60±2.35	72.77±0.45	78.47±2.5	68.69±0.54	75.40±0.82	18.95±2
March	0.27±2.6	74.75±0.89	74.43±0.25	74.83±0.65	77.57±0.45	67.68±0.7	77.39±0.45	18.13±1.5
April	0.61±2.5	73.36±0.95	71.67±0.45	72.21±0.68	70.64±0.58	75.24±0.36	75.88±0.56	24.36±0.95
May	79.85±2.45	75.30±0.56	72.42±0.18	75.15±0.75	72.79±0.85	76.73±0.85	76.45±0.32	25.55±0.45
June	73.61±1.45	73.36±0.75	70.67±0.45	71.21±0.41	71.64±0.75	78.42±0.28	74.88±0.43	21.64±0.85
July	75.48±0.23	78.03±0.56	72.42±0.45	78.52±0.52	75.79±0.68	75.27±0.48	77.55±0.41	22.55±0.61
August	75.28±0.56	78.38±0.58	71.54±0.25	71.90±0.62	71.92±0.69	70.25±0.49	78.33±0.34	23.77±0.68
September	69.45±0.75	77.26±0.79	75.13±0.45	75.38±0.67	73.85±0.45	70.64±0.5	72.26±0.62	24.36±0.24
7 Days								
October	54.04±0.58	61.12±0.64	61.90±0.5	45.31±0.5	54.82±0.65	61.03±0.86	55.95±0.76	19.35±0.6
November	51.76±0.35	64.69±0.57	48.69±0.5	52.09±0.4	43.23±0.57	46.79±0.92	61.88±0.45	20.37±0.4
December	59.73±0.56	55.90±0.36	59.88±0.54	47.18±1	52.50±0.68	63.82±0.56	54.52±0.37	23.56±0.48
January	57.83±0.65	59.01±0.28	52.74±0.35	65.49±0.9	47.60±0.54	54.08±0.65	49.17±0.59	23.46±0.45
February	55.94±0.65	67.89±0.68	54.94±0.75	46.76±0.5	62.60±0.57	57.66±0.43	57.76±0.34	16.86±0.69
March	52.23±0.25	50.50±0.36	54.17±0.56	65.06±0.58	45.73±0.43	56.93±0.31	51.93±0.86	15.61±0.45
April	65.24±0.58	63.11±1.38	63.99±0.62	62.31±0.46	57.81±0.35	57.90±0.58	52.59±0.6	18.05±0.66
May	47.29±0.69	5.41±0.4	54.23±0.62	46.79±0.68	53.85±0.5	60.47±0.55	53.86±0.5	21.48±0.45

Months	Treatment units							
June	50.89±0.84	63.82±1.26	56.04±0.58	45.50±0.86	55.38±0.55	48.47±0.45	51.59±0.58	19.05±0.9
July	52.48±0.83	63.30±0.25	50.42±0.42	67.12±0.58	60.88±0.47	52.27±0.35	64.55±0.64	12.45±0.9
August	54.29±0.34	50.00±0.58	52.33±0.36	64.21±0.79	58.40±0.4	54.36±0.46	58.31±0.46	15.33±0.5
September	49.64±0.54	51.94±1.5	51.57±1.8	62.00±0.95	54.44±0.6	52.73±0.6	65.19±0.66	17.73±1.89
14 Days								
October	82.93±0.65	89.17±0.56	82.40±0.16	82.06±0.39	87.12±0.26	81.12±0.35	85.63±0.65	33.56±0.66
November	73.31±0.2	85.42±0.5	69.79±0.54	81.09±0.6	72.14±0.36	80.01±0.56	84.26±0.6	35.13±0.56
December	82.24±0.45	88.81±0.5	82.81±0.3	80.10±0.6	86.37±0.56	78.68±0.36	82.69±0.6	30.66±055
January	82.72±0.36	77.90±0.45	75.94±1.2	78.38±0.6	82.08±0.65	75.44±0.54	80.31±0.57	36.50±0.46
February	85.85±0.75	76.12±0.5	84.17±2.5	78.08±0.64	71.65±0.76	80.35±0.43	81.03±0.45	31.42±1
March	76.35±0.36	83.88±0.45	89.48±2.6	82.56±0.66	81.60±0.64	84.67±0.56	85.25±0.5	32.20±1.2
April	87.18±0.46	84.29±0.8	85.83±2	84.79±0.92	80.08±0.56	87.44±0.35	82.31±0.63	31.05±0.59
May	80.42±0.26	81.85±0.5	74.38±2.6	85.79±0.56	82.08±0.36	87.02±0.62	85.15±0.64	33.01±0.5
June	83.76±0.6	85.34±0.62	77.51±0.56	77.91±1.2	88.60±0.23	82.74±0.36	80.03±0.46	31.42±0.45
July	78.78±1.5	79.42±0.86	76.51±0.38	81.91±1	87.65±0.86	84.74±1	86.52±05.3	30.42±0.57
August	87.78±1.2	76.13±0.69	80.95±0.85	75.91±0.8	86.65±0.36	81.35±0.45	86.22±0.56	29.94±0.5
September	78.65±1.6	80.42±0.2	85.95±0.37	80.08±0.69	82.46±0.36	83.74±0.5	88.22±0.35	34.73±0.95

Table 5.7 Percent removal of NO_3^--N throughout the year with respect to different treatment units and retention time (mean± SD)

Months	**Treatment units**							
3 Days	**Pi**	**Ph**	**T**	**Ph+T**	**Pi+T**	**Pi+Ph**	**Pi+Ph+T**	**Control**
October	67.75±0.4	60.64±0.46	71.05±0.74	61.40±0.25	66.06±0.26	57.07±0.75	61.28±0.75	13.91±0.65
November	68.51±0.75	65.86±0.5	59.94±0.26	64.54±0.45	78.85±0.45	57.70±0.15	62.08±0.48	25.14±0.85
December	65.09±0.68	64.37±0.65	66.18±0.15	66.61±0.75	62.44±0.52	53.92±0.32	61.87±0.61	37.70±0.45
January	62.19±0.58	58.21±0.45	63.67±0.55	55.92±0.6	62.19±0.36	53.67±0.45	60.82±0.48	13.88±0.65
February	56.58±0.36	49.95±0.35	56.53±0.45	57.45±0.45	60.15±0.85	57.14±0.45	59.80±0.5	10.31±0.64
March	62.31±0.6	54.83±0.15	66.39±0.52	61.13±0.3	62.03±0.36	52.09±0.62	55.78±0.41	11.97±0.85
April	53.88±0.56	61.27±0.25	56.68±0.32	57.34±0.48	62.70±0.45	58.39±0.42	59.44±0.36	17.18±0.45
May	52.81±0.45	61.83±0.45	62.96±0.56	60.90±0.3	75.43±0.52	64.52±0.26	61.95±0.31	25.00±0.62
June	45.19±0.66	61.40±0.45	56.64±0.23	57.49±00.15	63.83±0.25	57.39±0.64	60.09±0.42	26.79±0.75
July	59.29±0.57	65.60±0.45	55.49±0.45	43.79±0.26	62.93±0.45	68.97±0.32	54.01±0.62	12.09±0.65
August	51.21±0.57	54.34±0.58	58.19±0.32	45.71±0.9	50.60±0.5	59.78±0.31	54.29±0.58	7.69±0.85
September	60.71±0.6	61.34±1	56.75±0.52	50.53±0.25	52.55±0.15	56.38±0.45	48.74±0.34	24.81±0.85
7 Days								
October	63.72±0.45	54.64±0.35	59.59±0.5	56.94±0.26	63.72±0.85	74.18±0.41	61.84±0.45	14.90±0.64
November	69.50±0.48	84.75±0.58	56.90±0.56	40.99±0.45	70.16±0.65	82.10±0.45	74.80±0.85	11.15±0.45
December	64.03±0.65	67.69±1	68.87±0.56	64.16±0.25	73.71±0.45	73.32±0.25	66.38±0.95	20.21±0.75
January	68.27±0.45	65.35±0.35	64.61±0.46	62.76±0.5	57.49±0.36	61.70±0.52	56.24±0.62	25.42±0.61
February	63.17±0.95	67.19±1.5	68.81±0.14	69.88±0.55	68.50±0.5	72.93±0.85	67.13±0.42	28.08±0.35
March	60.67±0.3	65.88±0.45	62.49±0.45	60.85±0.45	66.63±0.45	68.61±0.42	58.43±0.61	33.32±0.5
April	61.06±0.48	63.64±0.36	71.67±0.52	72.12±0.35	71.36±0.6	76.74±0.62	63.79±0.32	25.64±0.85
May	58.85±0.65	66.03±0.36	70.42±0.5	72.15±0.45	70.79±0.48	77.27±0.32	64.55±0.42	32.55±0.42

Months	Treatment units							
June	50.43±0.8	64.75±0.75	54.27±0.52	61.83±0.36	65.57±0.75	67.98±0.6	60.87±0.94	19.33±0.62
July	66.14±0.45	70.34±0.35	72.53±0.85	70.33±0.36	74.49±0.25	76.95±0.85	63.40±0.15	33.08±0.75
August	64.48±0.5	68.12±0.45	66.07±0.45	70.28±0.3	64.86±0.45	70.31±0.23	61.05±0.64	29.49±0.65
September	68.76±0.55	70.22±0.55	66.67±0.6	65.28±0.45	61.86±0.52	71.08±0.5	57.53±0.45	32.49±0.75
14 Days								
October	87.57±0.35	81.30±0.45	88.22±0.58	82.70±0.48	86.16±0.45	88.24±0.26	83.86±0.45	25.54±0.75
November	91.52±0.45	94.39±0.68	82.72±0.45	77.47±0.26	92.84±0.78	93.36±0.34	91.05±0.35	33.39±0.64
December	87.44±0.4	88.49±0.89	89.47±0.62	88.03±00.6	90.12±0.75	87.70±0.42	87.18±0.4	50.29±0.64
January	75.44±0.34	80.49±1.5	78.47±0.25	82.03±0.25	80.12±0.25	87.70±0.42	73.18±0.85	30.29±0.95
February	73.78±0.6	76.18±2.1	75.95±0.56	72.85±0.45	70.65±0.45	80.74±0.45	64.03±0.45	35.42±1.5
March	78.30±0.56	80.79±0.78	74.54±0.58	72.25±0.45	70.84±0.85	82.63±0.6	69.75±0.26	32.54±0.25
April	74.05±0.36	76.08±0.28	73.16±1.45	70.38±0.62	68.81±0.25	73.38±0.85	62.31±0.51	31.05±0.45
May	70.86±0.46	72.19±0.68	66.16±0.25	74.32±0.68	71.37±0.25	80.48±0.42	67.27±0.64	37.46±0.45
June	75.26±0.46	78.33±0.82	73.13±1.2	72.93±0.48	68.33±0.5	89.50±0.32	75.75±0.85	32.25±0.64
July	76.76±0.75	77.34±0.45	74.95±1.5	81.91±0.65	78.65±0.62	85.74±0.61	71.22±0.46	36.42±0.67
August	75.65±0.63	77.81±0.64	70.95±1.2	75.08±0.78	76.65±0.45	82.74±0.42	76.52±0.75	38.42±0.87
September	77.95±0.58	85.34±0.48	75.40±0.25	81.25±0.25	80.36±0.45	84.81±0.42	71.63±.46	37.44±0.63

Table 5.8 Percent removal of NO_2^--N throughout the year with respect to different treatment units and retention time (mean± SD)

Months	**Treatment units**							
3 Days	**Pi**	**Ph**	**T**	**Ph+T**	**Pi+T**	**Pi+Ph**	**Pi+Ph+T**	**Control**
October	58.05±0.48	39.77±0.85	50.91±0.74	45.40±0.89	44.51±0.54	27.95±0.64	37.43±0.95	19.10±0.68
November	46.62±0.45	54.01±0.4	58.30±0.56	49.41±0.4	66.97±0.3	42.72±0.54	57.93±0.8	14.79±0.45
December	56.69±0.54	52.78±0.15	68.32±0.77	53.77±0.48	46.88±0.8	50.65±0.28	51.52±.74	21.09±0.84
January	52.53±0.84	55.19±0.64	42.88±0.48	43.57±0.48	42.92±0.3	52.90±0.48	67.21±0.36	13.92±0.6
February	63.62±0.8	55.58±.34	67.99±0.26	53.72±0.36	60.90±0.87	45.25±0.68	58.63±1.5	17.99±0.48
March	43.50±0.18	45.07±0.36	43.68±0.48	57.64±0.48	64.39±0.26	51.76±0.89	39.97±0.45	20.59±0.64
April	43.76±0.54	53.99±0.47	59.70±0.36	61.07±0.46	28.67±0.3	69.31±0.47	66.65±1.8	16.76±0.28
May	42.50±0.48	53.13±1	40.63±0.48	51.61±0.69	41.94±0.48	55.17±0.78	41.94±0.85	28.57±0.8
June	45.24±0.62	55.00±0.87	38.71±0.48	60.00±0.59	54.29±0.62	39.13±0.25	38.71±0.15	35.56±0.58
July	50.42±0.48	51.41±0.46	53.68±0.26	47.37±0.48	47.42±0.6	48.98±0.45	45.36±0.45	15.71±0.48
August	40.29±0.74	50.00±0.48	53.33±0.35	47.21±0.64	38.40±0.48	54.36±0.64	49.23±0.62	15.83±0.5
September	51.74±0.48	41.88±0.49	53.68±0.48	37.04±0.59	47.42±0.78	48.98±0.68	41.00±0.8	15.71±0.47
7 Days								
October	61.65±0.25	42.12±0.45	58.12±0.48	60.71±0.78	58.12±0.6	59.06±0.48	60.00±0.48	23.76±0.64
November	47.30±0.25	43.63±0.6	30.15±0.65	31.37±0.6	33.82±0.53	43.63±0.65	38.73±0.64	14.22±.25
December	27.25±0.6	41.80±1.5	38.57±0.5	43.42±0.56	51.50±0.4	59.59±0.45	40.19±0.6	33.72±0.3
January	61.06±0.15	62.71±1	55.76±0.55	53.65±0.5	67.53±0.54	54.35±0.6	55.06±0.48	19.65±0.6
February	67.14±0.54	37.41±0.5	59.69±0.48	56.00±0.48	62.82±0.48	60.63±0.64	61.57±0.48	17.49±0.48
March	53.76±0.45	50.08±0.48	65.63±0.36	60.41±0.36	62.86±0.64	56.53±0.48	61.31±0.64	4.54±0.6
April	56.98±0.36	56.53±0.26	59.18±0.58	60.86±0.48	56.08±0.3	64.92±0.69	60.34±0.61	17.76±0.5
May	35.34±0.5	48.27±0.3	48.27±0.6	49.89±0.3	49.89±0.8	53.12±0.74	49.89±0.6	32.10±0.48

June	32.10±0.5	35.34±0.48	49.89±0.78	35.34±0.48	43.42±0.48	62.82±0.5	49.89±0.6	27.25±0.68
July	52.09±0.38	54.34±0.36	56.19±0.36	45.71±0.64	50.60±0.64	59.78±0.65	54.29±0.28	12.92±0.5
August	45.19±0.5	61.40±0.6	56.64±0.3	57.49±0.3	63.83±0.3	57.39±0.85	50.09±0.35	22.86±0.74
September	58.86±0.6	63.33±0.48	61.39±0.58	65.24±0.5	57.58±0.48	60.41±.48	54.45±0.48	18.97±0.84
14 Days								
October	80.77±0.82	69.06±0.78	78.66±0.48	78.55±0.47	81.23±0.64	79.60±0.48	82.56±0.78	34.40±0.48
November	73.01±0.48	74.39±1.5	74.18±0.15	74.99±0.47	75.68±0.68	75.94±0.64	76.41±0.45	25.04±0.64
December	63.90±0.57	73.06±2.5	71.44±0.36	74.67±0.68	73.60±0.54	78.98±0.47	69.28±0.85	50.96±0.48
January	67.72±0.84	68.48±0.8	64.13±0.8	62.76±0.61	57.49±0.85	69.03±1	60.36±0.39	25.19±0.64
February	65.67±0.48	69.03±0.54	65.49±0.2	62.85±0.47	60.63±1.5	68.61±0.29	59.33±0.48	31.23±0.75
March	65.30±0.48	70.89±0.95	67.54±0.15	72.25±0.6	68.84±1.6	72.32±0.85	67.18±0.61	29.54±0.68
April	68.48±0.64	71.30±0.48	70.42±0.88	72.15±0.48	70.88±0.54	77.73±0.83	66.55±0.75	27.45±0.6
May	60.48±0.48	66.03±0.47	65.42±0.29	62.15±0.25	70.79±0.3	77.27±.46	64.55±0.84	32.55±1.5
June	67.85±0.58	71.03±0.75	72.42±0.48	70.15±0.95	72.88±0.73	67.27±0.48	64.55±0.47	25.55±1.6
July	62.48±0.48	65.12±0.48	56.07±0.47	60.28±0.43	64.29±0.64	72.31±0.64	62.05±0.68	27.95±0.47
August	59.64±0.48	63.33±0.64	61.39±0.64	65.24±0.35	57.58±0.85	68.13±0.8	58.47±0.47	25.97±0.58
September	63.14±0.61	65.34±0.49	56.53±0.8	58.53±0.49	55.55±0.18	66.38±0.2	52.74±0.95	24.81±0.39

5.4 Conclusion

Transformation of organic matters and cycling of nutrients in the natural environment employs numerous extracellular enzymes that are liberated by diverse microbial inhabitants. Consequently, estimation of the action of such enzymes having the potential to degrade organic matter and mineralize the nutrients inside the soil substrate is very vital. The vertical variation among the activity of several enzymes exhibited that the top layer of soil substrate possesses significantly maximum activity and significantly varied from the lower layer. The CWM unit established with *Pistia stratiotes* and *Phragmites karka* exhibited maximum activity in both top as well in deeper layer for the majority of the enzymes. Though, the temporal variation revealed significant differences over time and showed maximum activity in the months of May, June, and October for the majority of enzymes throughout the study. The correlation results among the activity of several enzymes and contaminants removal efficacy expressed that the phosphatase activity has positive and moderate correlation with TP and SRP removal respectively. Activity of urease and removal of NH_4^+-N was also positively correlated at the majority of the time with significant positive correlation in CWM units Pi+Ph and Pi+Ph+T. A moderate positive and negative correlation was also observed between urease activity and NO_2^--N and NO_3^--N exclusion. Elimination of BOD exhibited a positive correlation with the activity of DHA, FDA and MBC on 7 and 14 d retention times respectively. From these results, it is explained that extracellular enzymes and their action inside the wetland systems play critical roles in transforming and eliminating several pollutants from DW.

Chapter 6

Integration of CWM technology with UASB and FAB reactors for post-treatment of DW

6.1 Introduction

Developing countries have created capacities to treat only 8% of their total wastewater generated partially or fully and the rest of wastewater is discharged into water bodies which is loaded with a high concentration of nutrients, organics, and other hazardous substances (Worku et al., 2018; Ashekuzzaman et al., 2020; Kumar et al., 2020a; Alayu et al., 2021). Rapid urbanization, modernization and various agricultural activities have led serious threats to the available freshwater reserves by pollution loads all over the world (Kumar and Dutta, 2019b). The enormous growth in the use of organic chemicals for domestic purposes poses huge challenge for sewage treatment plants (STPs) in order to advance desirable treatment efficacy. It is reported that the wastewater generated from communities in India contains high loads of organics, nutrients and infectious pathogens (Bohdziewicz and Sroca, 2006; Jan and Pandit, 2013). The use of the aerobic process for the treatment of municipal wastewater especially for the exclusion of nutrients and residual organic requires high energy and operational cost, needs chemicals, generates secondary pollutants and skilled staff to operate expensive computerized treatment units (Badejo et al., 2014). The anaerobic methods for wastewater treatment containing a high concentration of organics are treated as sustainable techniques as compared to aerobic methods because of low energy requirement and generation of valuable resources such as organic fertilizers and biogas (Arantes et al., 2017; Alayu et al., 2021). Nearly 793 UASB based STPs are installed globally displaying the extensive use of this technology. Approximately half of these STPs are working in the subtropical and tropical regions (Fang and Liu, 2001). The UASB based STPs have been extensively used for the treatment of DW in Brazil, Africa, Columbia and India (Vassalle et al., 2020). Currently, in India, about 200 UASB reactors are utilized to treat industrial and municipal wastewater in various states. It is also reported that 80% of total worldwide installed UASB reactors for municipal wastewater treatment are presently operational in India (Khan et al., 2015; Engida et al., 2020). The main reason towards the selection of this method for municipal wastewater treatment is low energy requirements, low capital cost and lower O&M cost. However, both processes alone cannot be seen as providing 'complete' ecological resolution as their effluents always do not fulfill the discharge criteria mainly with respect to nutrients, residual carbon, pathogens and HMs (El-Khateeb et al., 2009; De la Varga et al., 2013; Tufaner, 2015;

Alayu et al., 2021). For that, it is suggested to employ a consequent post-treatment system together with wastewater treatment plants to protect the receiving water bodies and environment (Vassalle et al., 2020). Therefore, integration of CWM technology with anaerobic treatment methods such as UASB and FAB are gaining great momentum as an environmentally friendly wastewater polishing technology. Recently, various types of CWMs have been applied with dissimilar anaerobic reactors for wastewater polishing (Zeb et al., 2013; Jamshidi et al., 2014; Eginda et al., 2020; Kumar et al., 2020c). Additionally, the application of CWMs together with anaerobic treatment methods has noteworthy advantages for resource-scarce nations to manage their wastewaters with additional benefits (El-Khateeb and El-Bahrawy, 2013). Pre-treatment of wastewater with anaerobic methods may reduce the area of CWMs by 30 to 60%, avoid energy and chemical use, reduce HRT, increase resilience by eliminating clogging problem (Alvarez et al., 2008; Zeb et al., 2013; Ayaz et al., 2015). Pre-treatment through anaerobic methods follows step-by-step process via microbial degradations into CH_4, CO_2 and other trace elements (Menzel et al., 2020). Whereas, the CWMs remove various contaminants such as organics, nutrients, suspended solids, metals and infectious pathogens (De la Varga et al., 2013) by several physical and biochemical methods as post-treatment facility (Vymazal, 2007). Several studies have been conducted on CWMs as post-treatment facilities with anaerobic processes treating different types of wastewaters with efficient performance (De la Varga et al., 2013; Zeb et al., 2013). It is reported that CWMs with single planting of *Typha latifolia* and *Cyperus alternifolius* in a post-treatment system exhibited excellent removal efficacies for organics and nutrients from different types of wastewaters (Ciria et al., 2005; Tadese, and Seyoum, 2015; Sa'at et al., 2017; Gebeyehu et al., 2018). However, the use of macrophytes in combination may enhance the removal performance by augmenting oxygen supply, growing higher biomass and nutrient uptake and microbial activity (Rezaie and Salehzadeh, 2014; Geng et al., 2017; Kumar et al., 2020a). The removal efficiency of CWs varied significantly depending upon local environmental situations, design, macrophyte types, substrate materials and several working parameters. Therefore, the implementation of CWs for selected wastewater and prevailing environmental conditions needs local investigations to evaluate the overall performance of CWs planted with dissimilar macrophytic species. Thus, the main objective of this work is to assess the performance of full-scale UASB based STPs in single as well as in integration with CWM technology planted with different macrophytes for the DW treatment.

6.2 Material and Methods

6.2.1 Working of CWM units

All the detail regarding the design and operation of CWM units have been provided in the material and methods section under the sub-heading description of CWM units within chapter 3.

6.2.2 Sample collection and their analysis

The sampling and analysis of effluents discharged from different CWM units were carried out as provided in chapter 3.

6.2.3 Collection of Data

The performance of CWM units towards the removal of pollutants was observed within the laboratory of the department. The removal efficiency of CWM units was recorded at different HRTs. The total experiment time was the same as explained in chapter 3. Therefore, the characteristics of DW were also the same. However, here the removal efficacy data of various contaminants is based on only two HRTs. It is observed that the 14 days HRT is much higher when integrated with these treatment systems because of the requirement of space and storage of large volume of wastewater. However, the performance of UASB and FAB was evaluated based on the two years' data obtained from the Uttar Pradesh Jal Nigam and other different sources such as publications, articles and reports published.

6.2.4 Statistical Analysis

All the data presented here were analyzed using SPSS and Microsoft office excel. One-way ANOVA analysis was done to evaluate the variance among mean removal efficiencies with respect to retention times and CWM units.

6.3 Results and Discussion

6.3.1 Removal efficacy of CWM units

The removal efficiency of CWM units varied significantly depending upon the type of CWs and contaminants, retention time, type of macrophyte and substrate materials and several environmental factors such as pH, DO and temperature. The removal efficiency of BOD ranged from 50.86- 60.41% between different CWM units after 3-day retention time. However, the efficacy increased up to 81.38% with an increase in retention time (7 d) for CWM unit Pi+Ph (Fig. 6.1). Maximum removal capability for TP and SRP (60 and 58.46%, 77.42 and 73.60% for 3 and 7 days respectively) was also exhibited by the CWM unit Pi+Ph (Fig. 6.2 and 6.3). The removal was principally due to the adsorption, precipitation as well as utilization by macrophytes and available microbial populations (Engida et al., 2020). The removal efficiency for NH_4^+-N varied from 51.44- 77.89% among different CWM units with maximum removal by Pi+Ph (63.12%) and Ph (77.89%) at 3 and 7 days respectively (Fig. 6.4). Maximum removal capability for NO_3^--N and NO_2^--N was also expressed by CWM unit Pi+Ph (55.94-77.26 %) and (55.17-69.06 %) at both retention times (Fig 6.5 and 6.6). The removal of nitrogen may be due to the assimilation and uptake by macrophytes and microbial populations existing in CWMs. This study was supported by our previous research (Kumar et al., 2020a) and several others such as Khan et al. (2011, 2015), Engida et al. (2020).

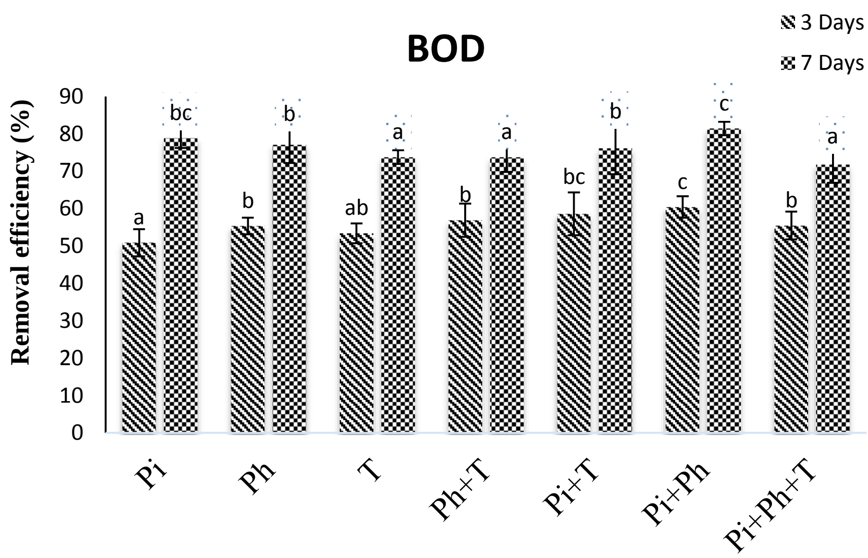

Fig. 6.1 Removal efficacy of BOD through different CWM units at two retention times (mean ±SD, n= 24). Different letters on the top of error bars represent significant variations among the mean removal efficiencies of various CWM units

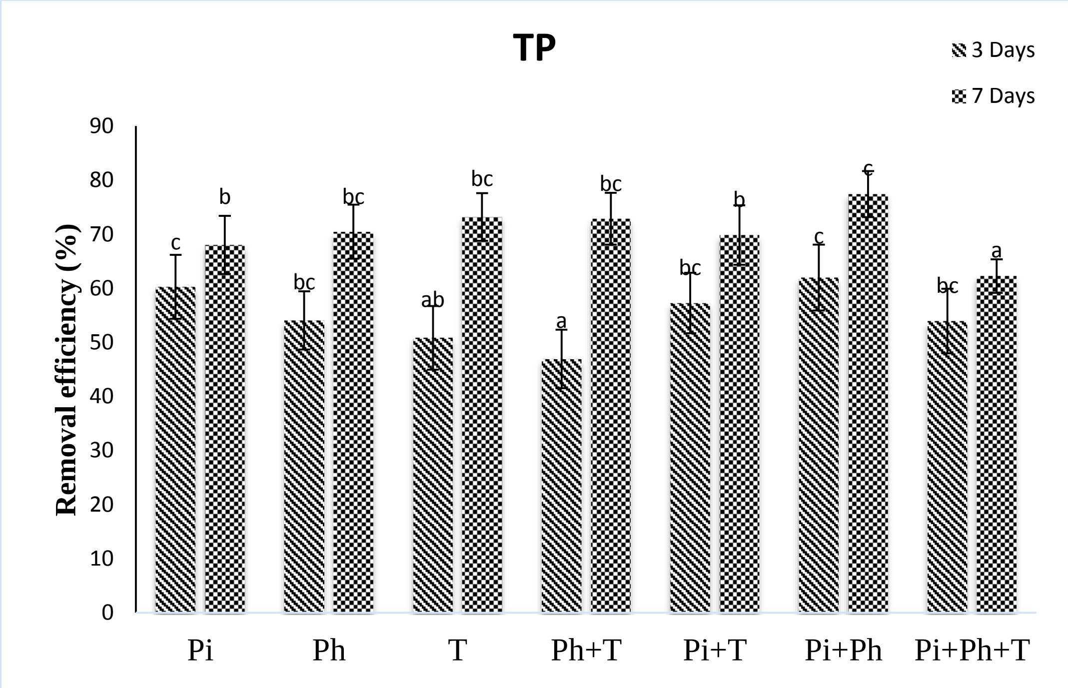

Fig. 6.2 Removal efficacy of TP through different CWM units at two retention times (mean ±SD, n= 24). Different letters on the top of error bars represent significant variations among the mean removal efficiencies of various CWM units.

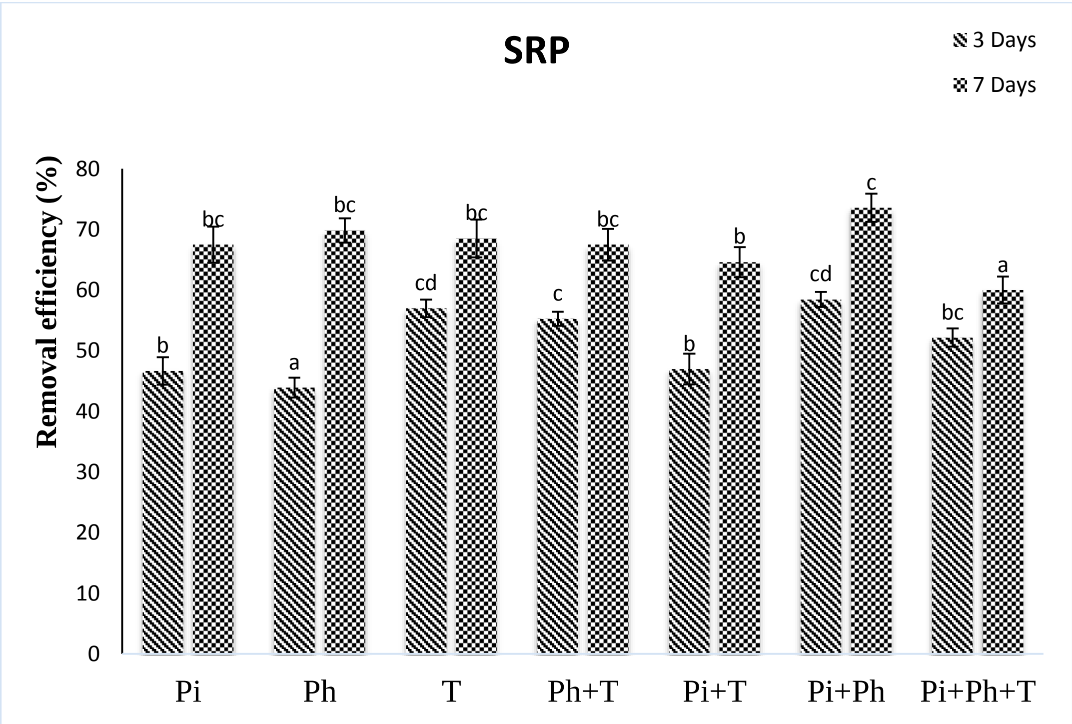

Fig. 6.3 Removal efficacy of SRP through different CWM units at two retention times (mean ±SD, n= 24). Different letters on the top of error bars represent significant variations among the mean removal efficiencies of various CWM units

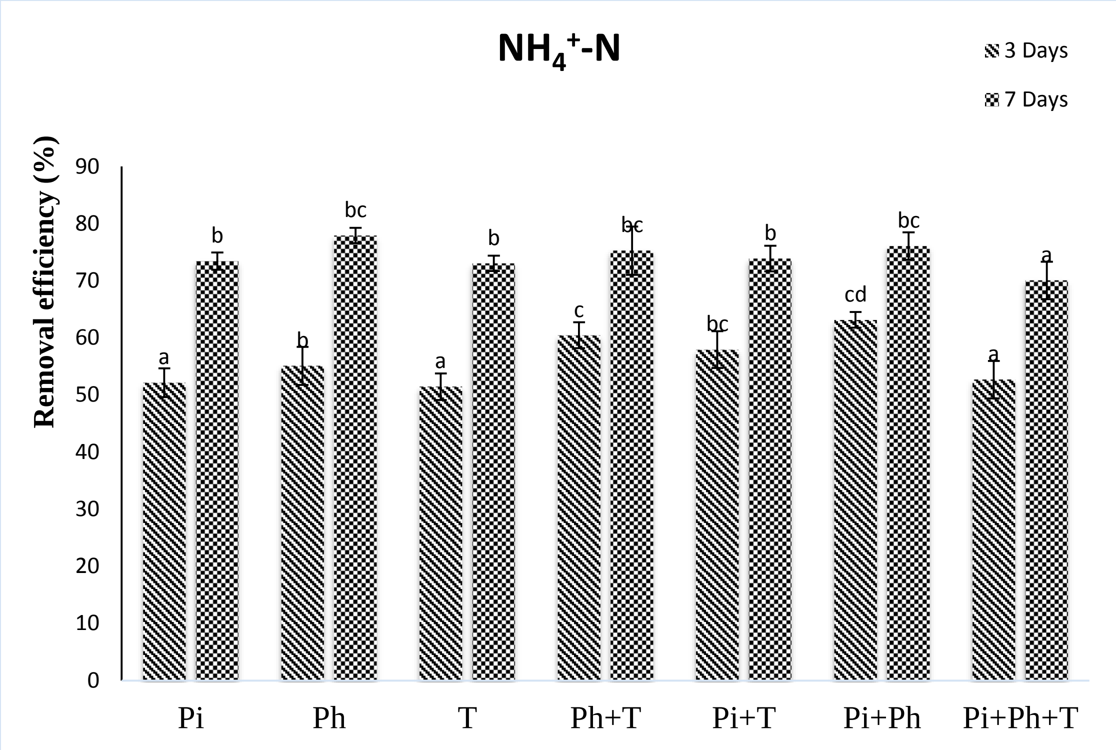

Fig. 6.4 Removal efficacy of NH_4^+-N through different CWM units at two retention times (mean ±SD, n= 24). Different letters on the top of error bars represent significant variations among the mean removal efficiencies of various CWM units

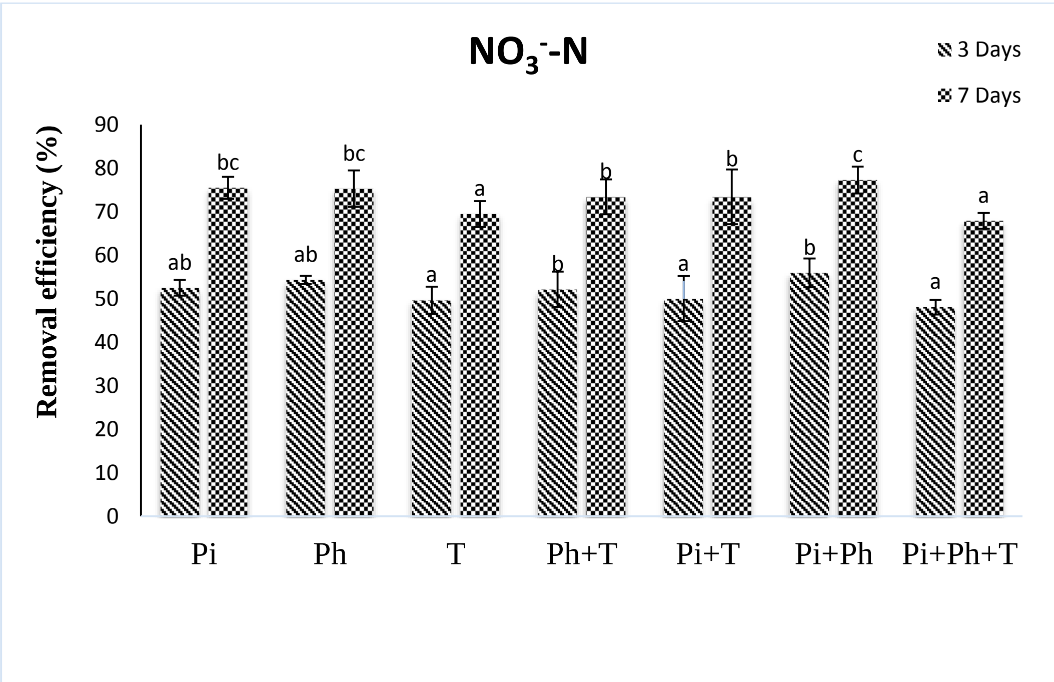

Fig. 6.5 Removal efficacy of NO_3^--N through different CWM units at two retention times (mean ±SD, n= 24). Different letters on the top of error bars represent significant variations among the mean removal efficiencies of various CWM units

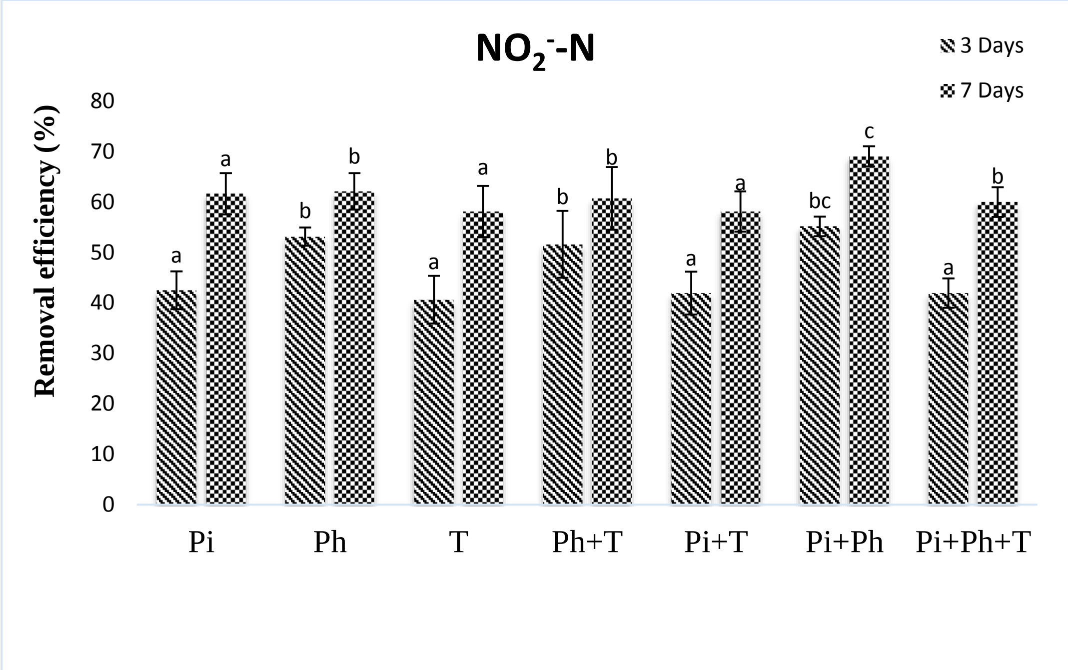

Fig. 6.6 Removal efficacy of NO_2^--N through different CWM units at two retention times (mean ±SD, n= 24). Different letters on the top of error bars represent significant variations among the mean removal efficiencies of various CWM units

6.3.2 Integration of CWs as a post-treatment system

The UASB based STPs have been extensively utilized for the treatment of DW in Brazil, Africa, Columbia and India (Vassalle et al., 2020). The main aim of selecting this method for the treatment of sewage is its low energy requirements, low O&M costs and sustainable operation. However, both processes alone cannot be seen as 'complete' ecological solution as their effluents always do not fulfill the discharge criteria for nutrients residual carbon, pathogens and heavy metals. The major wastewater treatment technology utilized for the treatment of DW throughout the world is UASB based STPs (Engida et al., 2020). The main problems associated with anaerobic digesters are the necessity of long HRTs for solids removal, extended start-up period, inadequate exclusion of organic matters, pathogens and nutrients and impure biogas. Consequently, investigators throughout the world are trying to establish new technologies to improve the efficacy of anaerobic digesters (Chong et al., 2012). However, the removal capability of UASB and FAB-based STPs do not always meet the discharge standards (Fig. 6.7).

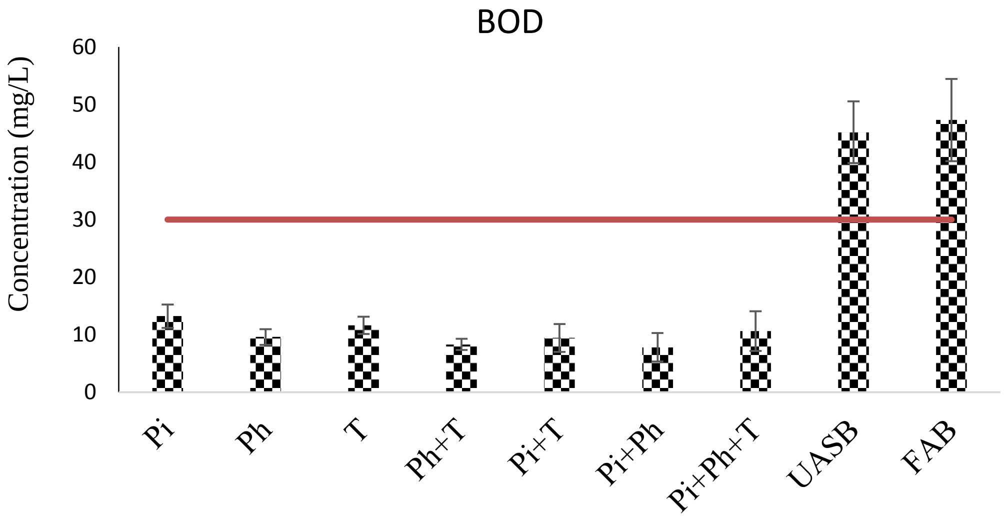

Fig. 6.7 Comparative removal efficiency of BOD from different CWM units along with UASB and FAB technology

The removal efficacy of UASB reactors for several wastewater contaminants is enhanced significantly by integrating them with other available wastewater treatment technologies. Several described treatment technologies utilized for the treatment of domestic wastewater are not economically viable, eco-friendly and sustainable. However, integration of CWs with available domestic wastewater treatment technologies may give the best alternative towards others due to its cost-effectiveness, environmental friendliness and sustainability (Tufaner, 2016 & 2020). The removal capability of UASB reactors in integration with CWs is higher, in terms of BOD (Upto 98 %) and FC (99.99), as compared to other available treatment technologies from domestic wastewater. However, the removal of COD (90%), TSS (92%), TN (89%), NH_4^+-N (70%) and TP (88%) were also observed optimum as reported in previous studies (Comino et al., 2013; Khan et al., 2015; Matos et al., 2017; Engida et al., 2020; Tufaner, 2020) (Fig. 6.8 and 6.9). The hydraulic loading rates of UASB and CWs were 3-6 hours and 10 days respectively.

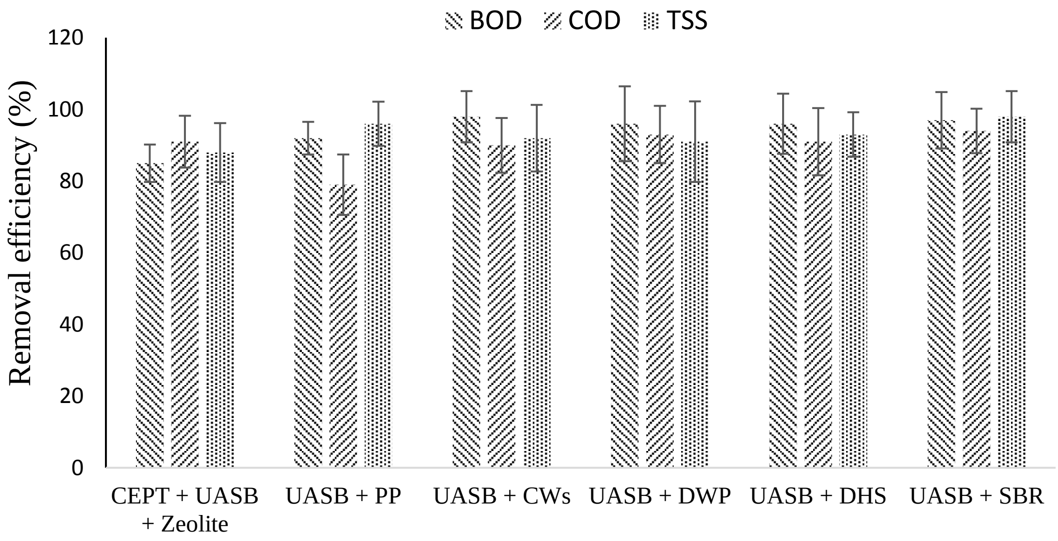

Fig. 6.8 Removal efficiency of UASB based STPs integrated with several other wastewater treatment technologies including CWs system for BOD, COD and TSS (mean± SD)

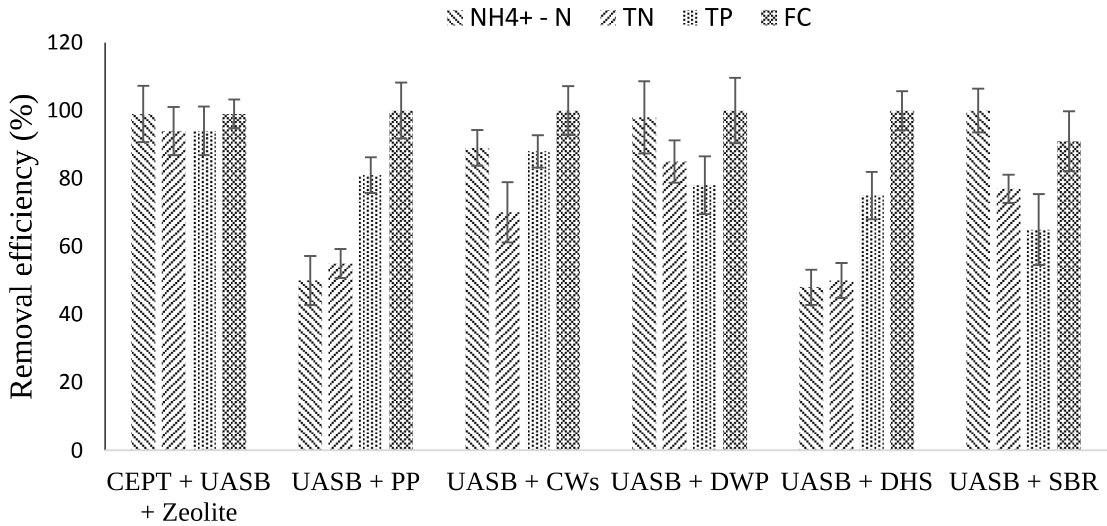

Fig. 6.9 Removal efficiency of UASB based STPs integrated with several other wastewater treatment technologies including CWs system for NH_4^+-N, TN, TP and FC (mean± SD)

*CEPT= Chemically enhanced primary treatment
*PP = Polishing Ponds, *CWs = Constructed Wetlands, *DWP= Duckweed Pond
*DHS= Down-flow hanging sponge, *SBR= Sequential batch reactor

The removal efficiency of UASB reactors for several wastewater contaminants working across India varies significantly. The data collected from various published articles exhibited that maximum removal of BOD was recorded in Noida (79 ± 0.89%) having the treatment capacity of 34 MLD followed by the Kanpur (72 ± 1.41%). However, the highest removal of COD and TSS (75 ± 1.2 and 70 ± 0.87 respectively) was exhibited by the UASB reactor working at Vadodara, Gujrat (Table 6.1). Such high removal efficacies of the UASB reactors may be due to their moderate input of wastewater in terms of volume, proper management and effective functioning of the reactors. However, the minimum removal efficiency was observed in Surat, Gujrat and Ludhiana, Punjab largely due to their improper management and operation of reactors (Table 6.1).

Table. 6.1 Treatment efficiency of some UASB based STPs throughout India

STPs Location	**Capacity (MLD)**	**% Removal**		
		BOD	COD	TSS
Agra	78	48 ± 2.3	43 ± 2.3	41 ± 4.21
Noida	27	53 ± 1.02	41 ± 3.45	59 ± 3.15
Noida	34	79 ± 0.89	51 ± 2.18	54 ± 4.12
Saharanpur	38	60 ± 2.15	55 ± 1.64	60 ± 3.1
Ghaziabad	56	60 ± 2.6	45 ± 2.4	-
Ghaziabad	70	60 ± 1.52	45 ± 3.64	-
Kanpur	-	72 ± 1.41	-	
Mirzapur	-	63 ± 1.13	-	-
Karnal, Haryana	40	60 ± 0.65	62 ± 2.51	54 ± 0.95
Vadodara, Gujrat	43	62 ± 0.98	75 ± 1.2	70 ± 0.87
Surat, Gujrat	100	47 ± 3.85	42 ± 5.4	40 ± 5.12
Ludhiana, Punjab	111	66 ± 1.92	59 ± 3.1	54 ± 6.23
Ludhiana, Punjab	152	59 ± 5.2	55 ± 4.21	49 ± 4.12
Ludhiana, Punjab	48	45 ± 6.12	29 ± 7.15	51 ± 2.01

The removal capacity for DW has been enhanced significantly by integrating the CW system with UASB reactors. Several UASB based STPs together with polishing ponds have been setup in many cities of India within the Yamuna Action Plan (Table 6.2). Such integrated systems worked efficiently to enhance the removal capability of several pollutants. The polishing pond was designed as a post-treatment facility in this system having 24 hours of retention time with 1.25 m water depth).

Table 6.2 Performance of some UASB based STPs under Yamuna Action Plan (YAP) (MOEF, 2006)

Location	Capacity (MLD)	Removal Efficiencies					
		BOD		COD		TSS	
		UASB	UASB+ PP	UASB	UASB+ PP	UASB	UASB+ PP
Gurgaon	30	61-69	62-72	61-68	63-81	66-73	67-80
Ghaziabad Trans	56	57-64	65-72	58-79	66-81	59-70	64-81
Ghaziabad Cis	70	53-66	64-76	69-78	65-88	64-73	67-78
Faridabad Zone-II	45	45-57	70-77	58-67	72-81	57-69	64-77
Faridabad Zone-III	50	51-67	65-78	66-73	69-83	71-78	70-84

Table 6.3 Performance of UASB reactors treating industrial and DW in various countries (Bula, 2014; Engida et al., 2020)

	Country	India	Japan	Brazil	Netherlands	Ethiopia	Cambodia
	Capacity	5 MLD	1148 L	106 L	6 m^3	700 m^3	35 m^3
	Temp.	25	-	21-25	20	-	23-24
	HRT	10	6	4.7	18	17	5.2
Influent (mg/L)	COD	590	532	265	550	2676	475
	BOD	167	240	150	-	1505	-
	TSS	-	-	123	-	686	225
Effluent (mg/L)	COD	201	197	133	165	228	170
	BOD	60	79	59	-	98	-
	TSS	-	-	33	-	96	65
Removal efficiency (%)	COD	66	63	50	70	91	66
	BOD	67	67	61	-	93.4	80
	TSS	-	-	73	-	82.6	69

The UASB based STPs have been extensively used for the treatment of DW in Brazil, Africa, Columbia and India (Vassalle et al., 2020). The exclusion efficacy of some UASB reactors working in different countries for the treatment of DW has been given in table 6.3.

6.4 Conclusion

The UASB based STPs have been extensively used for the treatment of DW in several countries including India. Currently in India, about 200 UASB reactors are utilized to treat municipal and industrial wastewaters. Around 80% of the total worldwide installed UASB reactors for municipal wastewater treatment are operational only in India. The removal efficacy of UASB reactors for several wastewater contaminants is enhanced significantly by integrating them with CWMs as post-treatment facilities. In this study, we have estimated that the concentration of BOD in the effluent of different CWM units after 3 days retention time successfully meets the discharge criteria of inland surface water. The maximum removal performance of several contaminants was expressed by the CWM unit Pi+Ph. However, the maximum removal of BOD via UASB reactor was recorded in Noida (79 ± 0.89%) having the treatment capacity of 34 MLD followed by the Kanpur (72 ± 1.41%). The UASB or FAB-based reactors alone have not achieved water quality up to the discharge standards. However, the removal capability of the UASB reactor in integration with CWMs in terms of BOD reached the highest (Upto 98%) as compared to other available treatment technologies from DW. The removal of COD (90%), TSS (92%), TN (89%), NH_4^+-N (70%) and TP (88%) were also observed optimum.

Conclusion

The presence of excess nutrients in wastewater causes eutrophication within the receiving waterbody by excess algal growth resulting into algal blooms. However, HMs within the environment are priority contaminants amongst the huge number of harmful elements due to their persistence and bioaccumulation ability. HMs, with high densities, are originating naturally from construction materials, agriculture activities, industrial processing and transportation. However, in DW they originate from household activities, small industrial operations and urban runoff. The majority of the HMs pose toxicity even at lesser fractions and their concentrations in tissues over some time could be harmful to ecosystems and human health. The excess concentration of these HMs in the environment may cause toxicity to living beings that can disrupt metabolic functioning. The concentration of these HMs within DW varies among cities or even for the same city depending upon residential and commercial locations and runoff. CWMs are well recognized eco-friendly, sustainable and cost-effective solution for the treatment of DW especially in decentralized settlements where traditional treatment methods are not practicable. Several environmental and working parameters are vital for the smooth functioning of CWMs. It is also observed that macrophytes and substrate materials also play a critical role in the elimination of wastewater contaminants. Consequently, selection of suitable macrophytes and substrate material is necessary**.** However, microbial communities present in the rhizosphere zone attached as biofilms help in the breakdown of several pollutants.

7.1 Removal efficiency of several wastewater parameters including HMs

HMs are among the most distressing contaminants present in the environment, mainly due to their toxicity and bioaccumulation properties. They act as a precursor to many bio-chemical reactions in the environment. In this study, the removal efficiency of several wastewater parameters including HMs were studied at different retention times. For that, eight CWM units in a single as well as in combinations were designed with three macrophytes and different substrate materials. The exclusion efficacy of all selected wastewater parameters such as BOD, TP, SRP, NO_2^--N NO_3^--N, and NH_4^+-N and various HMs with their removal rates were studied at three HRTs. The HMs uptake capacity of selected macrophytes was also evaluated in terms of BCF, TF, ATCF and RCF. CWM unit designed using free-floating *Pistia stratiotes* and emergent macrophyte *Phragmites karka* expressed higher removal ability with advanced reaction rate for most of the wastewater contaminants including HMs. However, the highest reaction rate was recorded for Cr, Cd and Mn respectively throughout the experiment. The elimination percentages of all selected HMs in different CWM units extended from 43.80 to 63.67%, 75.92 to 92.07% and 82.17 to 98.58 % for 3, 7 and 14 d HRTs respectively. Maximum TF and BCF were expressed by *Pistia stratiotes* and *Phragmites karka* for Zn (0.69 and 1.69 respectively). However, the maximum RCF and ATCF were observed in *Pistia stratiotes* for Cu and Zn (0.35 and 0.10 respectively). The correlation studies between removal efficiencies of HMs and several other parameters for this most efficient CWM unit also exhibited significant variation depending upon retention time. All selected HMs expressed a significant positive correlation with As.

7.2. Interspecific competition among macrophytes and their impacts on the removal of contaminants

Interspecific competition among macrophytes to acquire nutrients, space and light is one of the most important aspects to determine their growth responses. Nevertheless, due to high inconsistency in competition among several macrophytes, the growth of various macrophytes planted in mixed culture during field scale application is still not clear. Hence, it is also desirable to assess the interspecific competition and relative growth rate among macrophytes and their impact on the performance of CWMs. Several growth-related parameters of macrophytes such as the number of macrophytes, the total number of macrophytes, total dry biomass production, AGB, BGB and root length were also studied to distinguish the dominant nature of the macrophytes. *Typha latifolia* was recognized as a superior competitor in competition with both *Phragmites karka* and *Pistia stratiotes* due to their aggressive competitor nature that constrains the growth and development of adjoining macrophytes in mixed culture. *Phragmites karka* was identified as the superior competitor when planted with *Pistia stratiotes* and inferior competitor with *Typha latifolia*. However, *Pistia stratiotes* observed as a weak competitor against both macrophytes. The negative CV of *Pistia stratiotes* with *Phragmites karka* and *Typha latifolia* explained that the overall biomass of *Pistia stratiotes* in monoculture was higher as compared to mixed culture with these macrophytes. Similarly, *Phragmites karka* displayed negative CV with *Typha latifolia*. It is also due to the higher biomass of *Phragmites karka* in monoculture as compared to mixed culture. The relative growth rate of *Typha latifolia* was approximately two times greater than that of *Phragmites karka* among all experimental CWM units. The CWM unit having *Pistia stratiotes* and *Phragmites karka* in mixed culture exhibited more efficient removal of almost all selected water quality parameters most of the time throughout the experimental period.

7.3 Activity of different extracellular enzymes within soil substrate and their relation with contaminants removal

Activity of enzymes within soil substrate is suggested as a significant formative factor to enhance water quality in CWMs. In this study, the activity of different extracellular enzymes was assessed in two soil substrate layers for different CWM units. The vertical and temporal variations among the activity of different enzymes and their relationship with contaminants removal efficacy within several CWM units were also studied. The outcome of this work exhibited that the enzymes activity and contaminants elimination efficacy differ greatly depending upon retention time, substrate depth, type of contaminants and CWM units. The vertical variation among the enzyme's activity exhibited that the top layer (0-10 cm) of CWM units have significant activity of all selected enzymes and varied significantly from the lower layer. However, temporal variation exhibited significant variations over time with higher activity in the months of May, June, and October for most of the enzymes. CWM unit planted with *Phragmites karka* and *Pistia stratiotes* showed higher values of enzymes activity in the top as well in the deeper layer for most of the enzymes. The correlation results showed that phosphatase activity was significantly linked with the exclusion of TP and SRP in most of the CWM units. The activity of urease was significantly and positively correlated with NH_4^+-N removal in CWM units Pi+Ph+T and Pi+Ph. The activity of urease was also observed to have both negative and positive associations with the exclusion efficacy of NO_2^--N and NO_3^--N in various CWM units. However, the activity of the DHA enzyme expressed a negative association with the removal efficiency of BOD excluding CWM units Pi+Ph and Pi+Ph+T. Elimination of BOD and (MBC) exhibited a negative association with each other in the majority of the CWM units. However, a moderate positive and negative relationship was observed between BOD removal and FDA. CWM units revealed significant differences in enzyme activities depending upon treatment time and the presence of macrophytes.

7.4 Integration of CWMs with other available treatment technologies as a post treatment facility

The major wastewater treatment technologies utilized for the treatment of domestic wastewater throughout the world are UASB and FAB based STPs. About 80% of total worldwide installed UASB reactors for municipal wastewater treatment are present only in India. However, both processes alone cannot be seen as ‘complete’ ecological solutions as their effluents always do not fulfill the required discharge standards mainly for nutrients residual carbon, pathogens and heavy metals. In this study, the treatment performance of UASB reactors in single as well as in integration with CWM technology for the treatment of DW was studied. It is also observed that the removal efficacy of UASB reactors for several wastewater contaminants is enhanced significantly by integrating them with CWMs as a post-treatment facility. The concentration of BOD in the effluent of different CWM units after three days retention time successfully meets the discharge criteria of inland surface water. However, the maximum removal performance of several contaminants was expressed by the CWM unit Pi+Ph. The maximum removal of BOD via UASB reactor was recorded in Noida (79 ± 0.89%) having the treatment capacity of 34 MLD followed by the Kanpur (72 ± 1.41%). From the study, it is observed that the UASB or FAB-based reactors alone have not achieved water quality up to discharge standards. Nonetheless, the removal capability of the UASB reactor in integration with constructed wetlands in terms of BOD reached the highest (upto 98%) as compared to other available treatment technologies from DW. However, the removal of COD (90%), TSS (92%), TN (89%), NH_4^+-N (70%) and TP (88%) were also observed optimum.

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Scientific Publications and Achievements

a. Research Articles

1. **Kumar, S.,** Nand, S., Pratap, B., Dubey, D., & Dutta, V. (2021). Removal kinetics and treatment efficiency of heavy metals and other wastewater contaminants in a constructed wetland microcosm: Does mixed macrophytic combinations perform better? *Journal of Cleaner Production*, 327, 129468 **(IF: 9.29)**.
2. **Kumar, S.,** Pratap, B., Dubey, D., & Dutta, V. (2021). Interspecific competition and their impacts on the growth of macrophytes and pollutants removal within constructed wetland microcosms treating domestic wastewater. *International Journal of Phytoremediation*, 1-12. **(IF: 3.21).**
3. **Kumar, S.,** Nand, S., Dubey, D., Pratap, B., & Dutta, V. (2020). Variation in extracellular enzyme activities and their influence on the performance of surface-flow constructed wetland microcosms (CWMs). *Chemosphere*, 251, 126377. **(IF: 7.08)**.
4. **Kumar, S**., & Dutta, V. (2019). Constructed wetland microcosms as sustainable technology for domestic wastewater treatment: an overview. *Environmental Science and Pollution Research*, 26(12), 11662-11673. **(IF: 4.22)**.
5. **Kumar, S.,** Pratap, B., Dubey, D., & Dutta, V. (2020). Removal of nutrients from domestic wastewater using constructed wetlands: assessment of suitable environmental and operational conditions. *Environmental Sustainability*, 3:341–352.
6. Dutta, V., Dubey, D., & **Kumar, S.** (2020). Cleaning the River Ganga: Impact of lockdown on water quality and future implications on river rejuvenation strategies. *Science of the Total Environment*, 743, 140756. **(IF: 7.96)**.
7. Dutta, V., **Kumar, S.,** & Dubey, D. (2021). Recent advances in satellite mapping of global air quality: evidences during COVID-19 pandemic. *Environmental Sustainability*, 1-19.
8. Dubey, D., **Kumar, S.,** & Dutta, V. (2021). Impact of nutrient enrichment on habitat heterogeneity and species richness of aquatic macrophytes: evidence from freshwater tropical lakes of Central Ganga Plain, India. *International Journal of Environmental Science and Technology*, 1-18. **(IF: 2.86)**.

9. Pratap, B., **Kumar, S.,** Purchase, D., Bhargava, RN., & Dutta, V. (2021). Practice of wastewater irrigation and its impacts on human health and environment: a state of the art. *International Journal of Environmental Science and Technology*, 1-16. **(IF: 2.86)**.

b. Book Chapters

1. **Kumar, S**., & Dutta, V. (2019). Efficiency of constructed wetland microcosms (CWMs) for the treatment of domestic wastewater using aquatic macrophytes. In *Environmental biotechnology: for sustainable future* (pp. 287-307). Springer, Singapore.
2. **Kumar, S.,** Pratap, B., Dubey, D., & Dutta, V. (2020). Microbial communities in constructed wetland microcosms and their role in treatment of domestic wastewater. In *Emerging Eco-friendly Green Technologies for Wastewater Treatment* (pp. 311-327). Springer, Singapore.
3. **Kumar, S.,** Pratap, B., Dubey, D., & Dutta, V. (2021). Integration of constructed wetland technology in urban settlements as decentralized wastewater treatment facility and treated water reuse in agriculture. Tylor and Francis group (*under publication*).
4. Kumar, A., Singh., A.K., & **Kumar, S**. (2021). MnP enzyme: Structure, mechanisms, distributions and its ample opportunities in biotechnological application. In *Bioprospecting of Microbial Diversity* (*under publication*).

c. Conference Papers

1. **Saroj Kumar** and Venkatesh Dutta. (2017). Presented a paper entitled "**Constructed Wetlands Microcosms: A Sustainable way to Treat Domestic Wastewater**" in 1st North Indian Science Congress (1st NISC) at Babasaheb Bhimrao Ambedkar University Lucknow on December 10-11, 2017.
2. **Saroj Kumar** and Venkatesh Dutta. (2018). Presented a paper entitled "**Constructed Wetlands Microcosms (CWMs): An Innovative approach to Treat Domestic Wastewater**" on the occasion of National Science Day Celebration and Seminar on the theme **"Fostering Scientific Temper for**

Welfare of Society and Surroundings", organized by Babasaheb Bhimrao Ambedkar University Lucknow on February 27- 28, 2018.

3. **Saroj Kumar** and Venkatesh Dutta. (2019). Presented a paper entitled "**Interspecific competition and their effects on nutrients removal in Constructed Wetland Microcosms treating Domestic wastewater"** on 106th Indian Science Congress held at Lovely Professional University Jalandhar, Punjab on January 3-7, 2019.
4. **Saroj Kumar** and Venkatesh Dutta. (2020). Presented a paper entitled "**Wetlands as a natural filter"** in the Workshop and Awareness Campaign on *Wetlands for a sustainable Urban Future: Can Wetlands and Smart Cities co-exist,* World Wetlands Day on February 2nd 2020.
5. **Saroj Kumar** and Venkatesh Dutta. (2020). Presented a paper entitled "**Variation among Extracellular Enzymatic Activity (EEA) and their influence on the Performance of Surface - flow Constructed Wetland Microcosms (CWMs)"** in International Conference on Environmental Sustainability: Innovations, Translational Dimensions and Way Forward organized by Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow on February 10-12, 2020.

d. Workshops/Conferences attended

1. Participated in **MANTHAN 2017** (A program of Department of Higher Education, MHRD, New Delhi) YEH INDIA KA TIME HAI with the theme of "**New India 2022: Prospects and Challenges**" and secured ***Consolation Prize***, organized by Babasaheb Bhimrao Ambedkar University, Lucknow on September 4th, 2017.
2. Attended 58th Annual Conference of **Association of Microbiologists of India & International Symposium on Microbes for Sustainable Development: Scope & Applications (MSDSA-2017)** at Babasaheb Bhimrao Ambedkar University, Lucknow on November 16 - 19th, 2017.
3. Participated in Awareness Programme on **"Role of Hydrology Project in Ground Water Management in Uttar Pradesh"**, organized by Central Ground Water Board Northern Region, Lucknow and Ministry of Water Resources, River Development & Ganga Rejuvenation, Government of India on March, 16th 2018.

4. Attended a national seminar on "**Changing Dimensions of Natural Resources and Sustainable Development",** organized by Department of Geography, Avadh Girls Post Graduate College Lucknow on 29 - 30th March, 2018.
5. Attended a workshop on "**Socio-environmental Dimensions of Rejuvenating River Gomti"** organized by the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow in association with Lokbharti on 23rd April, 2018.
6. Attended a seminar on "**Environmental Sustainability: Present Scenario and Future Aspects**" organized by Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow on January 10th, 2019.
7. Participated in workshop on "**People Participation: A step towards Ground Water Management in Uttar Pradesh**" held at Lucknow on January 29th, 2019.
8. Attended a one-day symposium on "**Science for the people and the people for Science,**" organized by BBAU, Lucknow on the occasion of national science day, February 28th, 2019.
9. Participated in a national workshop on "**Building National STI Policy System and Governance of Effective R&D Ecosystem",** organized by DST-Centre for Policy Research, BBAU, Lucknow held on March 5th, 2019.
10. Participated in a workshop on **"Environmental Hazards of Electronic Waste,"** organized by Department of Environmental Science, BBAU, Lucknow held on March 13th, 2019.
11. Participated in the workshop on **"Reducing our Water Footprints"** on the occasion of *Swachhata Pakhwada* organized by Babasaheb Bhimrao Ambedkar University, Lucknow on September 13th, 2019.
12. Participated in an "**International workshop on Technology- led Innovation and Sustainability"** organized by DST Centre for Policy Research, Babasaheb Bhimrao Ambedkar University, Lucknow on February, 26th 2020.
13. Participated in the Online Training Programme on "**Child Centric Disaster Risk Reduction**" Jointly Organized by Child Centric Disaster Risk Reduction Centre, National Institute of Disaster Management, Ministry of Home Affairs, Govt. of India in Collaboration with Central University of Jharkhand held from 10-12th November 2020.

14. Participated in webinar on **WATER CONSERVATION STRATEGIES AND CHALLENGES** organized by Department of Environmental Science, Sir, Aurobindo College, University of Delhi under the aegis of Internal Quality Assurance Cell 7th September, 2020.
15. Participated in webinar on **LOW-COST RENEWABLE ENERGY DRIVEN (LC-RED) WASTEWATER TREATMENT OPTIONS** conducted on 20 February 2021.
16. Participated in the online training Programme on "**Climate Change and its Possible Impacts on Women and Children**" organized by National Institute of Disaster Management, Ministry of Home Affairs, Govt. of India in collaboration with Central University of Jharkhand on 21 July 2021.

e. Awards/Honors

1. **Research Excellence Award- 2020** by the *Institute of Scholars (InSc)* for the work entitled "*Variation in extracellular enzyme activities and their influence on the performance of surface-flow constructed wetland microcosms (CWMs)*". Chemosphere, 251, 126377.

f. Memberships

1. Life member of Indian Science Congress Association (ISCA- L41187)
2. Life member of Institute of Scholars (InSc id: 20200896).

g. Extracurricular activities

1. Participated in "**Annual Sports Festival- 2017"** and stood **1st** in *Interdepartmental Cricket tournament*, organized by Babasaheb Bhimrao Ambedkar University, Lucknow from April 07-10, 2017.
2. Participated in "**Annual Sports Festival- 2018"** and stood **1st** in *Interdepartmental Cricket tournament*, organized by Babasaheb Bhimrao Ambedkar University, Lucknow from March 05-15, 2018.
3. Participated in "**Annual Sports Festival- 2018"** in *Interdepartmental Volleyball tournament*, organized by Babasaheb Bhimrao Ambedkar University, Lucknow from October 25- November 05, 2018.

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Removal kinetics and treatment efficiency of heavy metals and other wastewater contaminants in a constructed wetland microcosm: Does mixed macrophytic combinations perform better?

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ABSTRACT

Eight constructed wetland microcosms have been designed using various combinations of three different macrophytes in single as well as mixed planting units. The removal efficiency of major water quality parameters such as biological oxygen demand, total phosphorus, soluble reactive phosphorus, ammonium, nitrate and nitrite-nitrogen and heavy metals was evaluated at three retention times. The potential of selected macrophytes for heavy metals uptake was also studied in terms of bioconcentration factor, translocation factor, aerial tissue concentration factor and root concentration factor. Constructed wetland microcosm unit designed using *Pistia stratiotes* and *Phragmites karka* exhibited higher removal potential with maximum decay constants for most of the heavy metals and other wastewater contaminants. Maximum translocation and bioconcentration factors were also expressed by *Pistia stratiotes* and *Phragmites karka* for Zn (0.69 and 1.69 respectively). However, the maximum root concentration factor and aerial tissue concentration factors were observed in *Pistia stratiotes* for Cu (0.35) and Zn (0.10) respectively. The paper reports significantly higher removal of wastewater contaminants including heavy metals in mixed species planting as compared to single species. The removal efficiencies for all metals studied in different constructed wetland microcosm units ranged from 43.80 to 63.67%, 75.92–92.07% and 82.17–98.58% for 3, 7 and 14 days retention time respectively. Relative higher values of decay constants were observed for Cr, Cd and Mn. However, the correlation studies between removal efficiencies of heavy metals and several other parameters exhibited significant variation over time.

1. Introduction

Constructed Wetland Microcosms (CWMs) are natural wastewater treatment systems that utilize physical, chemical and biological processes, widely known for their effective and eco-friendly treatment mechanism (Kumar et al., 2020; Wu et al., 2020; Zhang et al., 2020). A variety of aquatic macrophytes have been extensively studied for their efficacy towards wastewater treatment and used in the removal of almost all types of wastewater contaminants, including heavy metals (Khalifa et al., 2020; Zheng et al., 2020). Heavy metals affect the efficiency of macrophytes by stressing their early growth and development (Batool and Saleh, 2019). Due to the lack of appropriate treatment technologies, several industrial units and domestic settlements in developing countries discharge wastewater containing heavy metals and a number of contaminants directly into nearby waterbodies with little or no treatment (Kumar and Dutta, 2019). These metals cause severe damage to aquatic life and human health through accumulation in sediments and leaching in the groundwater affecting the freshwater ecosystem (Ali et al., 2019). Many other wastewater treatment techniques such as nanotechnology, membrane filtration and bioreactors have been evaluated for heavy metals removal from wastewater using a variety of environmentally friendly materials (Bavandpour et al., 2018).

Nevertheless, due to high operation and maintenance expenses and high energy requirements, an efficient and cost-effective onsite-treatment method remains a serious challenge to both the researchers and practitioners throughout the world. In developing countries like India, it is a common tendency to mix industrial waste with domestic and agricultural runoff (Kumar et al., 2020). Such intercourse of domestic

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sewage and industrial effluent containing heavy metals enhances the complexity and cost of treatment (Yadav et al., 2012). To avoid the mixing of heavy metals into freshwater ecosystems, there is a rapidly growing concern for the treatment of mixed wastewater in urban as well as rural localities (Ding et al., 2018; Zhang et al., 2020). However, earlier findings revealed that CWMs have numerous drawbacks when operated for removal of heavy metals. The removal efficiency differs significantly ranging from 20% to more than 90%, based on the design of CWMs, type of macrophytes, substrate and metal species (Gill et al., 2014; Nuamah et al., 2020). From these findings, it is estimated that the toxicity of metals on microbial development and macrophytes growth might influence the removal potential and biodegradation of organics and nutrients (Kumar and Dutta, 2019). The performance of CWMs towards metals removal is studied through retention time, precipitation and co-precipitation, macrophyte uptake, and microorganism's metabolism (Marchand et al., 2010; Vymazal and Březinová, 2016). These are influenced by several aspects such as pH, type of substrate, characteristics of wastewater and macrophytic species (Sochacki et al., 2014). Amongst them, the selection of substrate is the most significant factor as it expressed the absorption and adsorption abilities of metals in CWMs (Wang et al., 2020a). Gravel is an extensively used substrate material in CWMs and attains wide-ranging removal efficacies of cadmium, lead, copper and several other wastewater contaminants (Sizirici et al., 2018). Further, lava rock and bio-ceramic are two common substrate materials that offer a higher surface area and permeability, higher adsorption capability and long-term durability (Katukiza et al., 2014). It is reported that bio-ceramics and geolite have significant removal potential towards copper and cadmium and other wastewater contaminants, and rocks from the volcanic eruption are also gaining substantial investigation attention due to their higher absorption capacity (Wang et al., 2020b). The presence of macrophytes within CWMs has significant positive effects on metals removal (Gill et al., 2014). It is reported that rhizofiltration might be the primary route for metals uptake from wastewater (Rezania et al., 2016). However, the role of macrophytes planted with different substrate materials in a CWMs for metals removal is still indefinite. The translocation factor (TF) and bioconcentration factor (BCF) are two vital parameters that are used to assess the capability of macrophytes for metal phytoremediation (Katukiza et al., 2014). BCF signifies the metal uptake ability of plants, whereas TF characterizes the internal transportation of metals from roots to both leaves and stems (Zhang et al., 2020). In this study, eight CWM units have been designed in a single as well as in mixed culture to study the most efficient macrophytic combinations for the removal of heavy metals and several other contaminants from domestic wastewater at three retention times (3, 7 and 14 days respectively). Biomass of different macrophytes (roots, stems and leaves) were also analyzed to study the capability of particular macrophytes for the maximum heavy metals uptake. The pseudo second-order kinetics were used to assess the decay rate of heavy metals from effluent medium.

2. Material and methods

2.1. Description of site

Eight CWM units were designed at Babasaheb Bhimrao Ambedkar University, Lucknow (26.7697° N, 80.9262° E) UP, India in concrete containers with dimension 1.2 × 0.60 × 0.76 m (L * W * D) as a batch experiment. All CWM units were filled with 8 × 8 × 16 cm crushed stone (diameter 2–4 cm), sand and soil respectively as substrate materials as defined in several earlier studies (Shao et al., 2014; Kumar et al., 2020, 2021). *Pistia stratiotes* (*Pi*), *Phragmites karka* (*Ph*) and *Typha latifolia* (*T*) each with similar weight/length were planted in single as well as in combination with a primary density of 18 macrophytes per unit (Kumar et al., 2020). The equal proportions of these three species were used in mixed CWM units such that the density of total macrophytes was 18 macrophytes per unit initially. The eight CWM units on the basis of macrophytes were designated as: *Pi* (*Pistia stratiotes*), *Ph* (*Phragmites karka*), *T* (*Typha latifolia*), *Ph* + *T* (*Phragmites karka* + *Typha latifolia*), *Pi* + *T* (*Pistia stratiotes* + *Typha latifolia*), *Pi* + *Ph* (*Pistia stratiotes* + *Phragmites karka*), *Pi* + *Ph* + *T* (*Pistia stratiotes* + *Phragmites karka* + *Typha latifolia*) and an unplanted unit as control. Selected macrophytes were reported as having significant metal uptake capacity in several previous studies in different parts of the world. These emergent and free-floating macrophytes are regularly used in wetland systems to treat almost all types of wastewater in several continents such as Europe and Asia (Rezania et al., 2016; Kumar et al., 2020, 2021; Zhang et al., 2020). The CWM units were then filled with tap water and left for one month for macrophytic stabilization. After the stabilization of plants, the raw domestic wastewater was collected in a sterile plastic drum from a wastewater drain that received domestic sewage, transported at site and filled in all CWM units equally. Each CWM unit held a total volume of 200 L of untreated domestic wastewater every month as suggested in several previous studies (Elfanssi et al., 2018; Kumar et al., 2020, 2021). The hydraulic retention times (HRTs) were 3, 7 and 14 days for this experiment and the total experimental time was one year from October 2018 to September 2019. The average concentration of selected physico-chemical parameters and heavy metals of raw domestic wastewater is presented in Tables S1 and S2 of the supplementary file 1 respectively.

2.2. Sample collection and their analysis

Effluent samples from each unit were collected in triplet after 3rd, 7th and 14th d of each month throughout the year in 500 mL plastic bottles. For the analysis of selected heavy metals such as Chromium (Cr), Cadmium (Cd), Lead (Pb), Zinc (Zn), Arsenic (As), Copper (Cu), Nickel (Ni) and Iron (Fe), the collected effluents samples were digested (except for As) on a hot plate using nitric acid and perchloric acid (5:1 ratio) to convert metal ions into their soluble salts (Mant et al., 2006). After that, the samples were analyzed through Thermo Fisher Scientific™ ICP-MS (iCAP TQ). Several other parameters such as Biological oxygen demand (BOD), Total phosphorus (TP), soluble reactive phosphorus (SRP), ammomium (NH_4^+-N), nitrate (NO_3^--N) and nitrite-nitrogen (NO_2^--N) were analyzed as per the methods prescribed by APHA (2017). The initial concentrations of heavy metals and other selected parameters have been given in Tables S1 and S2 of the supplementary file 1 respectively.

2.3. Macrophyte sample collection and their analysis

After 14 d retention time of each month, macrophytes samples were collected and separated into root and aerial parts. Biomass samples of different macrophytes and soil substrate were analyzed for heavy metals concentrations to calculate the bioconcentration factor (BCF), translocation factor (TF), root concentration factor (RCF) and aerial tissue concentration factors (ATCF) as described earlier (Mant et al., 2006). A reference macrophyte and experimental blank of soil substrate from an unplanted unit was also studied to minimize the error for background metal concentration and to validate the accuracy and precision of the sample digestion process and analytical method. Bioaccumulation of heavy metals in aerial parts and roots of macrophytes was investigated to understand the accumulation capability of particular macrophyte planted in various CWM units.

2.4. Statistical analysis

The statistical analysis for all the given data has been done using SPSS (version 20) and Microsoft Office Excel (version 2016). Analysis of variance (One-way ANOVA) was used to determine statistical differences among the decay constant and means of heavy metals and other wastewater contaminants removal from different CWM units at different retention times ($p < 0.05$). Correlation among the mean removal efficiencies of heavy metals and other parameters were also analyzed using Pearson correlation coefficient.

2.5. Calculation

The removal efficiency (RE) of each heavy metal was calculated as shown below:

$$RE\ (\%) = (1 - Ce/Ci) * 100 \quad (1)$$

Where C_e and C_i stands for effluent and influent concentration respectively

$$\text{Translocation factor (TF)} = C_A / C_R \quad (2)$$

Where C_A and C_R stands for concentration of heavy metals in aerial parts and roots of macrophytes respectively (Salem et al., 2014).

$$\text{Bioconcentration factor (BCF)} = (C_A + C_R)/C_S \quad (3)$$

Where, C_S represents heavy metal concentration in soil substrate (Soda et al., 2012).

$$\text{Root concentration factor (RCF)} = C_R/C_I \quad (4)$$

$$\text{Aerial tissue concentration factor (ATCF)} = C_A/C_I \quad (5)$$

Here, C_I represents the initial concentration of heavy metals in wastewater (Khan et al., 2009).

The pseudo second-order kinetic model was used to describe the effective metals decay rate in mg per liter of effluent medium per day ($mgL^{-1}\ d^{-1}$) and expressed as decay constant (k) (Zhang et al., 2020; Singh et al., 2021).

$$k = 1\ (A_0 - A)/(t * A * A_0) \quad (6)$$

Here, A_0, A and t represent initial and final concentration and time respectively.

3. Results and discussion

3.1. Environmental conditions

The annual average temperature ranged from 27 to 36.3 °C, relative humidity 66–90% and solar intensity was within the range of 101*100 to 512*100 Lux. Relative humidity (RH), solar intensity and temperature (T) were measured to know their daily variation. The average DO (dissolved oxygen) and pH of the raw domestic wastewater applied in this study during the experimental period were 1.20 ± 0.52 mg L^{-1} and 5.48 ± 0.52 respectively. The concentration of DO varied greatly among various treatment units due to different macrophytic combinations that favor the growth of different microbial communities (Zhang et al., 2010). All of the microbial populations present in CWMs consume DO for the breakdown of organic waste existing in domestic wastewater. Initially, the concentration of DO decreased rapidly due to aerobic respiration and chemical oxidation (Ding et al., 2018). After that, there was an increasing trend shown by several CWM units because most of the organic materials were oxidized and taken up by microorganisms. The unit planted with *Pi* + *Ph* acquired highest DO concentration among other treatment units throughout the experiment. However, the pH values also increased from 5.48 to 7.5 after 14 d of retention time. It is well known that the higher temperature favors the growth and activity of microorganisms that directly take part in the breakdown of several contaminants within the system (Kumar and Dutta, 2019).

3.2. Heavy metals removal by different CWM units

The results of one-way ANOVA ($p < 0.05$) indicated that there is a significant variance among removal efficiencies of different metals with respect to CWM units at three retention times (Fig. 1). The removal efficiencies for all metals studied in different CWM units ranged from 43.80 to 63.67%, 75.92–92.07% and 82.17–98.58% for 3,7- and 14 d retention time respectively. The maximum removal of Cr was exhibited by CWM units *Pi* + *Ph* and *Pi* + *Ph* + *T* (more than 57%) after 3 d and *Pi* + *Ph* (90 and 94.32%) after 7- and 14 d respectively. Maximum removal efficiency for Cd was shown in CWM unit *Pi* + *T* (56.69%) at 3 d treatment and *Pi* + *Ph* (90.36 and 95.92%) at 7- and 14 d respectively. The higher removal efficiency of Pb was revealed in CWM unit *Ph* + *T* (58.89%), *Pi* + *Ph* (about 91%) and *Pi* + *T* (94.13%) at 3, 7- and 14 d respectively. The maximum removal of Zn was expressed by CWM unit *Pi* + *Ph* (57.55 and 92.07%) for 3 and 7 d respectively. However, at 14 d the maximum removal efficiency was exhibited by the CWM unit designed using single macrophyte *Phragmites* (95.75%). Similarly, maximum removal of Cu was shown by CWM units *Pi* + *Ph* (56.35 and 89.55%) for 3- and 7 d retention time and for 14 d, maximum removal was shown by CWM units *Pi* + *Ph* and *T* (91%). The maximum removal of Mn was exhibited by CWM unit *T* (63.67) for 3 d and *Pi* + *Ph* (82.72 and 91.50%) for 7 and 14 d respectively. The highest removal efficiency for Fe was exhibited by CWM unit *Ph* + *T* (58.09%) and *Pi* + *Ph* (91.94%) for 3 and 7 d retention time. Similarly, in two other CWM units, namely *Pi* + *T* (91.87%) and *Pi* + *Ph* + *T* (91.02%) also almost same removal efficiency were observed. However, the maximum removal of Fe for 14 d retention time was exhibited by CWM unit *Ph* (98.14%). Removal of As showed a similar trend among different CWM units with the maximum in *Ph* + *T* (59.10%) and *Pi* + *Ph* (89.30 and 94.32%) for 3, 7 and 14 d retention time respectively. The maximum removal of Ni was recorded in CWM unit *Ph* + *T* (55%) and *Pi* + *Ph* (89.47%) for 3 and 7 d respectively. However, for 14 d, the maximum removal capability was observed in CWM units *Pi* + *T* (98.58%) and *Pi* + *Ph* (98.19%). Overall, the CWM unit designed using *Pistia stratiotes* and *Phragmites karka (Pi + Ph)* performed well for the removal of the majority of the metals at different retention times. The removal efficacies for all selected heavy metals at 7 and 14 d of retention time exceeded up to 92% and 98% respectively. The higher metal removal capacity of CWMs might be the outcome of the aerobic settings within these systems, in which metal cations co-precipitated into oxyhydroxides, hydroxides and ferric oxides (Vymazal and Březinová, 2016). Removal of metals in CWMs is a complex mechanism in which macrophytic uptake, together with biotic and abiotic responses play a crucial role in the elimination of metals through the process of phytoaccumulation, phytostabilization and phytovolatilization (Batool and Saleh, 2019). It is also known that the removal of heavy metals involves several chemical reactions such as precipitation and complexation which facilitate the attenuation of ions from the substrate materials (e.g. CO_3^{2-} and OH^-), microbial populations (e.g. $CO_3^{\ 2-}$) and macrophytes (e.g., organic acids) (Di Luca et al., 2011; Liu et al., 2007; Nuttall and Younger, 2000; Wang et al., 2020b). In the present study, there were almost constant removal efficiencies for the selected heavy metals with standard deviations less than 15.61% indicating the stable performance of CWM units vis-a-vis removal of heavy metals.

3.3. Removal kinetics of heavy metals

The exclusion kinetics of heavy metals in this research work were fitted with pseudo second-order kinetic model ($R^2 > 0.9$). For this, a plot for the heavy metals concentration in effluent medium vs. retention times was plotted and the decay constants for heavy metals mass from effluent medium were determined using model Eq. (6). The values of decay constants for several metals at different retention times in single as well as in mixed CWM units, have been exhibited in Supplementary Table S12 along with their initial and final concentrations. There is a significant difference between the decay constants with respect to retention times ($p < 0.05$). The relative higher decay constant was observed for Cr, Cd and Mn throughout the experiment as described by Zhang et al. (2020) for mixed industrial - domestic wastewater. With respect to CWM units, units planted with *Phragmites karka* and *Pistia*

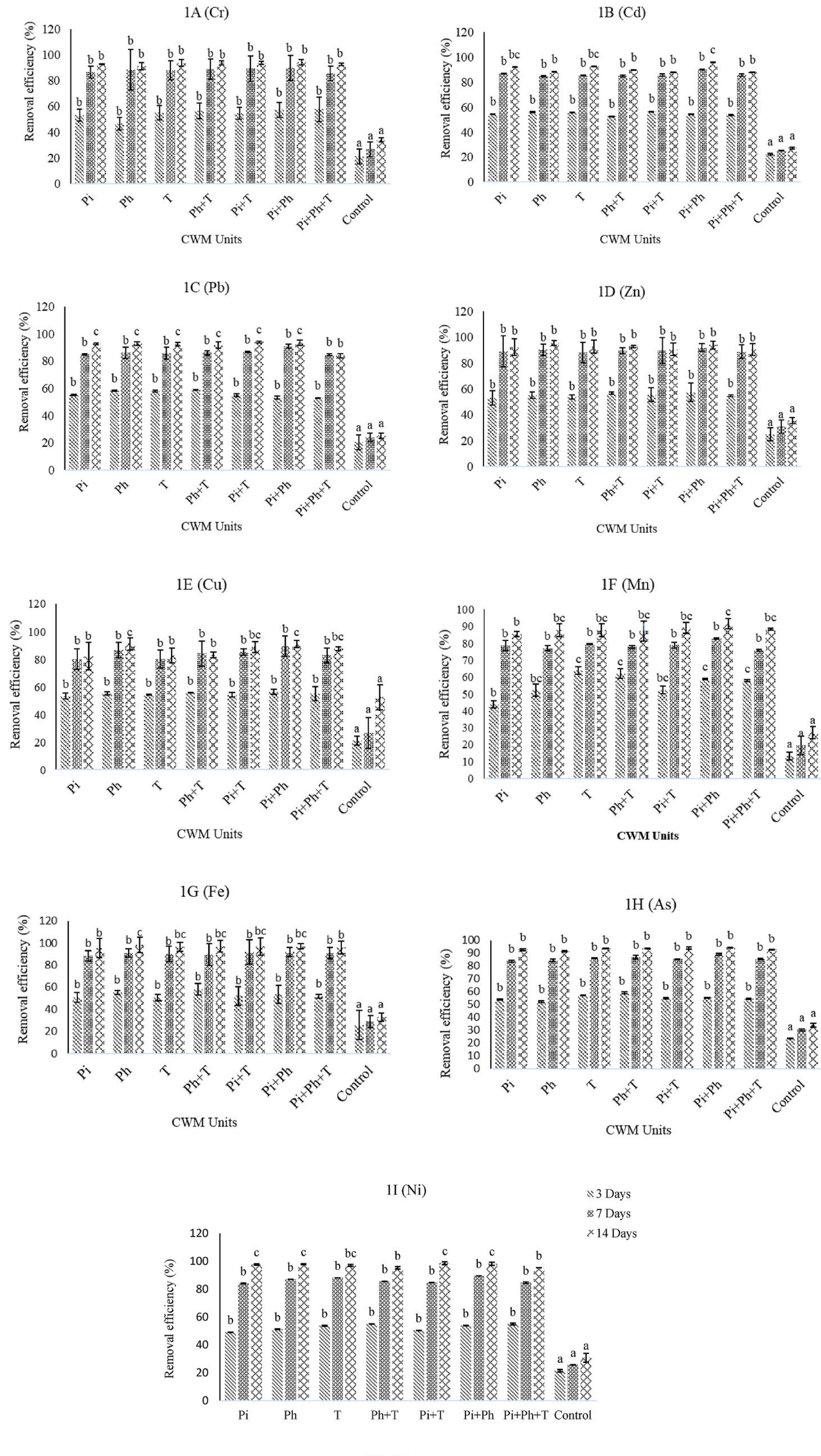

Fig. 1. A, B, C, D, E, F, G, H and I represents removal efficiency of different metals with respect to different CWM units and retention times. The standard deviation of the means (n = 12) has been represented as error bars. Different letters (a, b and c) represent variance among removal efficiencies for different CWM units ($P < 0.05$).

stratiotes exhibited higher decay constant as compared to others for most of the metals. It is known that in constructed wetlands, early adsorption is an effective removal mechanism for heavy metals (Zhang et al., 2020). Initially, decay of several heavy metals was higher (for 3 and 7 d) and then either increased slowly or maintained a relatively stable level (from 7 to 14 d) for the majority of metals in several CWM units (Yan et al., 2020). This is due to fast preliminary adsorption, followed by a slow macrophytic uptake. A similar pattern for heavy metals removal was described earlier, in which heavy metals, mainly Pb, Zn, Cu and Cd from wastewater, were adsorbed primarily by substrate materials (Di Luca et al., 2011; Sekomo et al., 2012). In the present work, it is observed that about 50–70% of heavy metals concentrations were removed by adsorption in substrate materials during the initial 3 d. However, the contribution of macrophytic uptake to the overall removal of heavy metals was relatively low (approximately 5–15%). The kinetics of heavy metals removal were evaluated to determine the degree of heavy metals removal with respect to time and for understanding the basic nature of removal processess through various macrophytes. These kinetic results may also be useful for enhancing the decay rate of heavy metals from domestic wastewater to improve the overall performance of CWMs and further to design and optimize the field-scale wetlands treatment process (Titah et al., 2019; Singh et al., 2021).

3.4. Concentration of heavy metals within the root and aerial parts of selected macrophytes

The concentrations of heavy metals within the substrate, roots and aerial parts of macrophytes along with their TF, BCF, RCF and ATCF have been provided in Tables 1–3 respectively. BCF is defined as the capability of macrophytes for the accumulation of heavy metals from the substrate material, while TF is a vital indicator utilized to evaluate macrophytes' potential and their feasibility for the phytoremediation process (Wu et al., 2011). Macrophytes with higher TF and BCF are known as the best agents for the phytoremediation process in the natural environment (Ndeda and Manohar, 2014). In this study, Maximum TF and BCF were expressed by *Pistia stratiotes* and *Phragmites karka* for Zn (0.69 and 1.69 respectively). However, the maximum RCF and ATCF were observed in *Pistia stratiotes* only for Cu (0.35) and Zn (0.10) respectively. Emergent macrophytes such as *Phragmites australis* and *Typha latifolia* have excellent growth characteristics and considered as hyperaccumulators for several metals (Salem et al., 2014; Yan et al., 2020). The efficient removal of Cr and Fe by *Phragmites karka* was shown in a study conducted by Mustapha et al. (2018). However, the accumulation of heavy metals and other contaminants in *Typha latifolia* might be due to their aggressive and robust competitor nature among others (Kumar et al., 2021). The majority of heavy metals are removed efficiently in the presence of these macrophytes through absorption via the root system (Scholz and Hedmark, 2010; Mustapha et al., 2018). The concentration of heavy metals deposited in the root was 10–100 times more as compared to aerial parts (leaves and stems) of *Phragmites* and *Pistia* in a horizontal flow CW (Vymazal and Březinová, 2016). Furthermore, certain heavy metals are translocated to aerial parts of the macrophytes. The translocation of heavy metals among shoots and roots is evaluated by several researchers (Lu et al., 2013; Gill et al., 2014; Rezania et al., 2016; Zhang et al., 2020). The bioaccumulation of heavy metals via macrophytes includes numerous steps; these are rhizosphere mobilization, translocation through root epidermis and cortex into the xylem (Kumar and Dutta, 2019). Translocation of metals via roots of macrophytes might be due to sequestration in vacuoles (Yan et al., 2020). Several ions such as oxalate, acetate, citrate and malonate are expelled via the root system as exudates that acts as chelators to bind metal ions (Ryan et al., 2001; Rezania et al., 2016). A non-stop flow process may assist in the mobilization of heavy metals from substrate materials into the rhizosphere region, which simplifies the macrophytes uptake (Zhang et al., 2020). The aerobic atmosphere formed in CWMs might also encourage the development of oxides (mainly Fe and Mn), hydroxides and oxyhydroxides that allows heavy metal exclusion by complexation as discussed earlier (Salem et al., 2014; Vymazal and Březinová, 2016).

4. Removal efficacy of other wastewater contaminants

Removal efficiency of BOD varied significantly among different CWM units and with varying retention times. Maximum removal performance was exhibited by CWM unit planted with *Phragmites karka* and *Pistia stratiotes* (*Pi* + *Ph*) at 3 and 14 d retention time. However for retention time of 7 d, the highest removal performance was exhibited by CWM unit containing all three macrophytes (Fig. 2).

Removal of TP varied from 46.93 to 87.44% depending upon various plant combinations and retention times. Maximum removal efficiency for TP and SRP was also shown by CWM unit planted with *Phragmites karka* and *Pistia stratiotes* at all times (Fig. 3). However, removal of SRP varied from 43.91 to 58.46%, 60–73.60% and 75.37–87.84% for 3, 7 and 14 d retention time respectively with maximum efficacy by CWM unit *Pi* + *Ph* (Fig. 4). It is reported that *Phragmites karka* and *Pistia stratiotes* are more effective towards the phosphate elimination (Yasar et al., 2018). The study was also supported by several previous researches conduted in various regions of the world (Saeed et al., 2018; Zhai et al., 2016; Kumar et al., 2020; Zheng et al., 2020).

The removal efficacy of NH_4^+-N ranged from 51.44 to 87.27% depending upon different retention times and CWM units (Fig. 5). The maximum removal efficacy for 3 d was exhibited by CWM unit planted with *Phragmites karka* and *Typha latifolia* and for 7 and 14 d, by single planting of *Phragmites karka* and *Pistia stratiotes* and *Phragmites karka* throughout the experiment respectively (Zhai et al., 2016; Hickey et al., 2018; Kumar et al., 2020).

Removal of NO_3^--N among different CWM units with three retention times varied from 48.08 to 55.94%, 67.89–77.26% and 78.65–85.42% respectively. The maximum removal efficacy was again exhibited by CWM units *Pi* + *Ph* (Fig. 6). The other CWM units also expressed optimum results as shown in Fig. 6. The similar results were observed for the NO_2^--N. The removal efficacy of NO_2^--N varied from 40.63 to 89.60% based on different CWM units and retention times (Fig. 7). The study was supported by Jampeetong et al. (2012), Kumar et al. (2020) and Kumar

Table 1
Concentration of heavy metals in substrate and aerial parts of *Phragmites karka*, respectively (n = 12, retention time 14 d).

Metals	Substrate mg kg^{-1}	Root mg kg^{-1}	Aerial part mg kg^{-1}	TF	BCF	RCF	ATCF
Cr	1.02 ± 0.01^{a}	0.14 ± 0.002^{a}	0.01 ± 0.001^{ab}	0.11 ± 0.01^{a}	0.15 ± 0.01^{bc}	0.12 ± 0.03^{a}	0.01 ± 0.001^{a}
Cd	0.75 ± 0.01^{a}	0.21 ± 0.003^{a}	0.01 ± 0.001^{a}	0.07 ± 0.01^{a}	0.30 ± 0.02^{ab}	0.19 ± 0.01^{b}	0.01 ± 0.002^{a}
Pb	4.02 ± 0.05^{a}	1.02 ± 0.05^{a}	0.03 ± 0.002^{a}	0.03 ± 0.02^{b}	0.29 ± 0.02^{ab}	0.20 ± 0.04^{b}	0.01 ± 0.002^{a}
Zn	10.21 ± 0.15^{ab}	4.05 ± 0.25^{ab}	1.19 ± 0.004^{ab}	0.52 ± 0.01^{ab}	1.69 ± 0.31^{d}	0.23 ± 0.01^{bc}	0.07 ± 0.001^{b}
Cu	1.73 ± 0.3^{a}	0.4 ± 0.008^{a}	0.1 ± 0.001^{a}	0.04 ± 0.01^{a}	0.25 ± 0.04^{ab}	0.17 ± 0.01^{a}	0.01 ± 0.003^{a}
As	1.03 ± 0.05^{a}	0.25 ± 0.006^{a}	0.02 ± 0.001^{a}	0.08 ± 0.001^{b}	0.26 ± 0.04^{ab}	0.17 ± 0.02^{a}	0.01 ± 0.004^{a}
Mn	2.94 ± 0.06^{ab}	0.87 ± 0.02^{a}	0.07 ± 0.002^{a}	0.09 ± 0.02^{bc}	0.37 ± 0.07^{cd}	0.22 ± 0.002^{bc}	0.02 ± 0.005^{a}
Ni	2.80 ± 0.04^{a}	0.5 ± 0.04^{a}	0.03 ± 0.001^{a}	0.06 ± 0.01^{a}	0.21 ± 0.06^{b}	0.14 ± 0.05^{a}	0.01 ± 0.001^{a}
Fe	14.07 ± 0.15^{a}	3.02 ± 0.85^{a}	1.50 ± 0.02^{a}	0.40 ± 0.05^{cd}	1.51 ± 0.2^{d}	0.15 ± 0.12^{a}	0.07 ± 0.002^{b}

Different letters a, b, c and d represent significant difference among mean concentrations of various metals in different plant parts at $p < 0.05$.

Table 2
Concentration of heavy metals in substrate and aerial parts of *Typha latifolia* respectively ($n = 12$, retention time 14 d).

Metals	Substrate mg kg^{-1}	Root mg kg^{-1}	Aerial part mg kg^{-1}	TF	BCF	RCF	ATCF
Cr	1 ± 0.02[a]	0.04 ± 0.006[a]	0.02 ± 0.001[a]	0.34 ± 0.02a	0.12 ± 0.01[a]	0.08 ± 0.01[a]	0.03 ± 0.02[a]
Cd	0.7 ± 0.01[a]	0.06 ± 0.005[a]	0.03 ± 0.02a	0.55 ± 0.04b	0.13 ± 0.01[bc]	0.07 ± 0.01[a]	0.04 ± 0.006[a]
Pb	3.25 ± 0.2[a]	0.72 ± 0.009[a]	0.04 ± 0.02[ab]	0.10 ± 0.07a	0.31 ± 0.01[c]	0.18 ± 0.04[a]	0.02 ± 0.005[a]
Zn	11.59 ± 0.2[ab]	3.02 ± 0.12[b]	1.03 ± 0.05[b]	0.32 ± 0.02a	1.27 ± 0.51[cd]	0.18 ± 0.02[b]	0.06 ± 0.004[b]
Cu	1.6 ± 0.03[a]	0.14 ± 0.004[a]	0.03 ± 0.001[a]	0.17 ± 0.07a	0.16 ± 0.03[b]	0.10 ± 0.01[a]	0.02 ± 0.003[b]
As	0.69 ± 0.05[a]	0.24 ± 0.006[a]	0.02 ± 0.02[a]	0.14 ± 0.06a	0.24 ± 0.04[a]	0.17 ± 0.02[b]	0.02 ± 0.001[a]
Mn	2.31 ± 0.4[a]	0.90 ± 0.001[a]	0.06 ± 0.02[a]	0.11 ± 0.32a	0.40 ± 0.02[bc]	0.23 ± 0.05[bc]	0.02 ± 0.002[a]
Ni	2.50 ± 0.6[a]	0.61 ± 0.005[a]	0.80 ± 0.03[a]	0.31 ± 0.08a	0.41 ± 0.02[cd]	0.17 ± 0.01[a]	0.05 ± 0.01[a]
Fe	12.17 ± 0.8[ab]	3.22 ± 0.85[ab]	1.39 ± 0.08[b]	0.46 ± 0.01bc	1.00 ± 0.62[b]	0.16 ± 0.04[a]	0.05 ± 0.03[b]

Different letters a, b, c and d represent significant difference among mean concentrations of various metals in different plant parts at $p < 0.05$.

Table 3
Concentration of heavy metals in substrate and aerial parts of *Pistia stratiotes* respectively ($n = 12$, retention time 14 d).

Metals	Substrate mg kg^{-1}	Root mg kg^{-1}	Aerial part mg kg^{-1}	TF	BCF	RCF	ATCF
Cr	0.80 ± 0.1[a]	0.13 ± 0.02[a]	0.03 ± 0.01[a]	0.29 ± 0.05	0.20 ± 0.06[a]	0.11 ± 0.02[b]	0.03 ± 0.002[a]
Cd	0.83 ± 0.03[a]	0.10 ± 0.03[a]	0.03 ± 0.01[a]	0.32 ± 0.04	0.15 ± 0.01[a]	0.09 ± 0.02[b]	0.03 ± 0.005[a]
Pb	3.5 ± 0.2[ab]	1.05 ± 0.09[ab]	0.21 ± 0.2[ab]	0.20 ± 0.02	0.51 ± 0.12[a]	0.20 ± 0.01[a]	0.04 ± 0.007[b]
Zn	11.70 ± 0.9[b]	2.32 ± 0.65[b]	1.6 ± 1.1[ab]	0.69 ± 0.06	1.60 ± 0.5[a]	0.15 ± 0.07[a]	0.10 ± 0.002[c]
Cu	1.1 ± 0.3[a]	0.61 ± 0.04[a]	0.05 ± 0.1[a]	0.08 ± 0.4	0.61 ± 0.05[b]	0.35 ± 0.05[a]	0.03 ± 0.001[a]
As	1.09 ± 0.02[a]	0.28 ± 0.006[a]	0.03 ± 0.02[a]	0.13 ± 0.03	0.29 ± 0.01[bc]	0.20 ± 0.05[b]	0.03 ± 0.004[a]
Mn	3.05 ± 0.5[a]	0.49 ± 0.08[a]	0.05 ± 0.3[a]	0.12 ± 0.05	0.22 ± 0.08[b]	0.14 ± 0.04[a]	0.02 ± 0.002[a]
Ni	2.83 ± 0.4[a]	0.4 ± 0.002[a]	0.06 ± 0.53[a]	0.17 ± 0.07	0.21 ± 0.05[a]	0.12 ± 0.06[a]	0.02 ± 0.006[a]
Fe	12 ± 1.25[b]	4.09 ± 0.95[b]	1.09 ± 1.52[ab]	0.27 ± 0.03	1.43 ± 0.57[b]	0.24 ± 0.02[a]	0.06 ± 0.01[b]

Different letters a, b, c and d represent significant difference among mean concentrations of various metals in different plant parts at $p < 0.05$.

et al. (2021). It is reported that the maximum nutrient uptake ability may be a characteristic of the macrophyte to accumulate more nutrients in their tissues as shown by *Pistia stratiotes* and *Phragmites karka* (Jampeetong et al., 2012).

5. Correlation among removal efficiency of heavy metals and other contaminants

The correlation between heavy metals removal efficiencies and other selected parameters for most efficient CWM unit (*Pi* + *Ph*) has been studied at different retention times. The correlation studies exhibited large variations depending upon different heavy metals, other wastewater contaminants and on retention times. The majority of the heavy metals showed significant positive correlation with As. The results of correlation studies have been provided in the Supplementary file 2. The significant positive correlation was observed between Cu and Ni, Cr and BOD, Ni with SRP, NH_4^+-N and NO_3^--N and Zn with NH_4^+-N. The positive correlations among some heavy metals may demonstrate that they may have close association, similar accumulation behaviours or originate from the same pollution source. (Titilawo et al., 2018; Agoro et al., 2020). In the present study, both NH_4^+–N and NO_3^-–N expressed positive correlations with majority of heavy metals ($p < 0.05$). It is reported that concentration of NH_4^+–N can dominate the elimination of heavy metals by macrophytes within CWs (Yin et al., 2018). Nevertheless, associated interaction mechanisms of NH_4^+–N and heavy metals are not fully explained yet. Furthermore, concentration of Cd could alleviate the negative impact of NH_4^+–N on the growth of macrophytes (Cui et al., 2021). However, the significant negative correlation was observed between Zn and Cd with BOD, TP, SRP and NO_2^--N with Cd and between NO_2^--N and As. Similar results were exhibited in the studies conducted by Agoro et al. (2020) and Zhang et al. (2020) for mixed domestic -industrial wastewater within CWs and by Mishra and Kumar (2021) for river water. The negative correlation of BOD with majority of the heavy metals suggests the inhibition of growth and activities of hetero and autotrophic microorganisms may be due to the heavy metal toxicity. High concentration of heavy metals can reduce the oxidation capabilities and several biochemical activities of these microorganisms resulting in deteriorated microbial biomass and diversity (Wang et al., 2018; Bhat et al., 2020). However the negative correlation of phosphorus with metals such as Cr and As may be due to the structural analogue that helps in mitigation of heavy metal toxicity. Phosphate and arsenate also share a common transportation pathway via roots of the macrophytes (Sayantan, 2017; Sayantan and Das, 2020).

6. Conclusion

The main aim towards conducting this research was to assess the removal kinetics and treatment efficiency of heavy metals and other wastewater contaminants in several CWM units with respect to combination of various macrophytes and different retention times. The removal efficiencies of different metals varied significantly depending upon the macrophytic combinations and retention times. CWM unit designed using *Pistia stratiotes* and *Phragmites karka* (*Pi* + *Ph*) performed well for the removal of majority of the heavy metals and also acquired higher DO concentration as compared to other units. Maximum TF and BCF were expressed by *Pistia stratiotes* and *Phragmites karka* for Zn (0.69 and 1.69 respectively). However, the maximum RCF and ATCF were observed in *Pistia stratiotes* for Cu (0.35) and Zn (0.10) respectively. The higher value of decay constants were observed for Cr, Cd and Mn as compared to other heavy metals studied. Correlation studies between removal efficiencies of heavy metals and several other parameters for most efficient CWM unit exhibited significant variation over time. All selected heavy metals expressed significant positive correlation with As. This study has an important design implication on constructed wetlands as combination of emergent and free-floating macrophytes in the mixed culture could outperform the single species constructed wetlands.

CRediT authorship contribution statement

Saroj Kumar: Methodology, Data curation, Writing – original draft, Writing – review & editing, Project administration, Investigation,

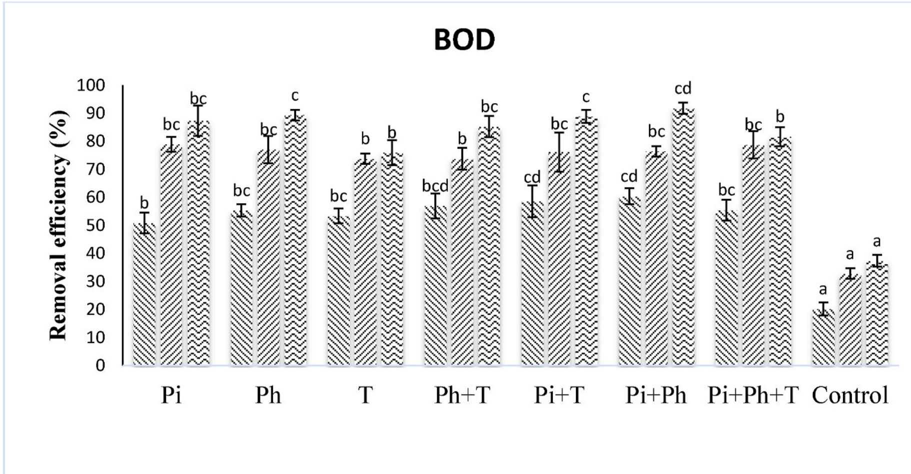

Fig. 2. Removal efficiency of BOD by different CWM units at three retention times (mean ± SD). Different letters as a, b, c and d represent significant variations at $p < 0.05$.

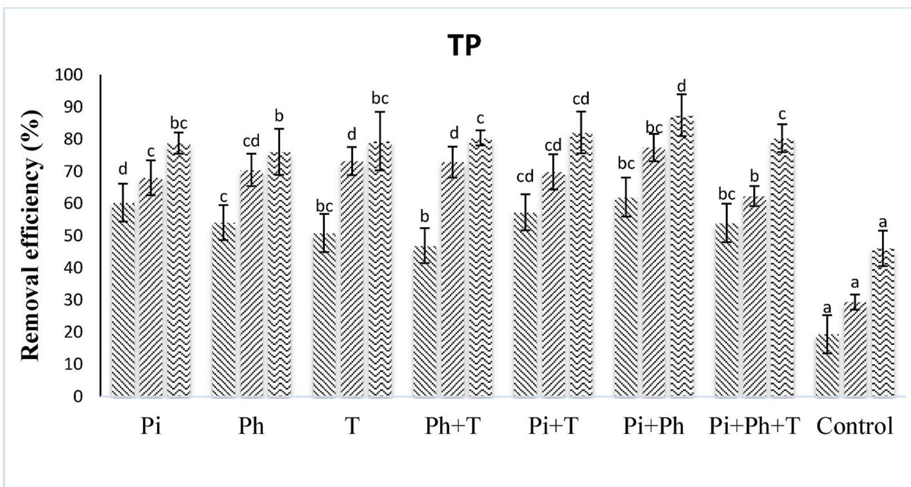

Fig. 3. Removal efficiency of TP by different CWM units at three retention times (mean ± SD). Different letters as a, b, c and d represent significant variations at $p < 0.05$.

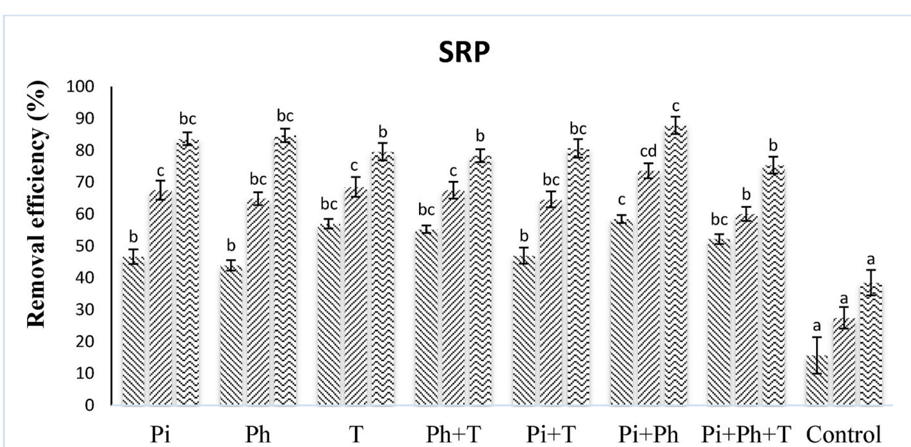

Fig. 4. Removal efficiency of SRP by different CWM units at three retention times (mean ± SD, n = 24). Different letters as a, b, c and d represent significant variations at $p < 0.05$.

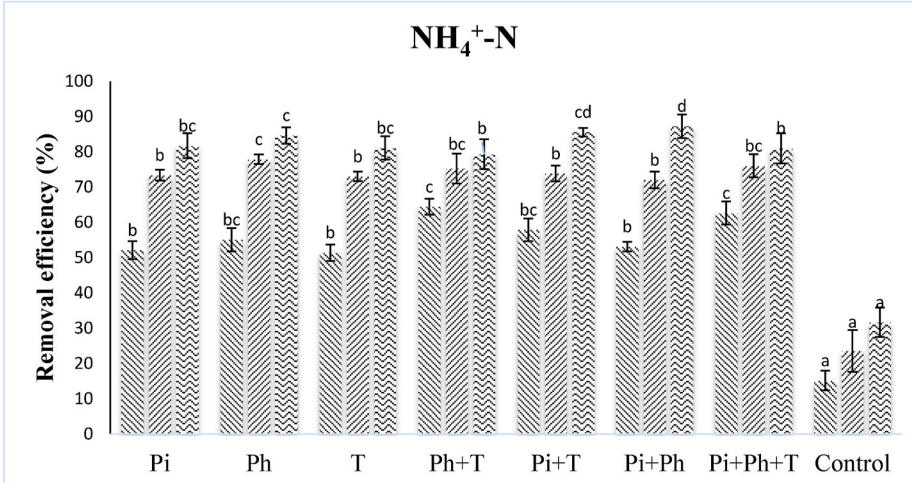

Fig. 5. Removal efficiency of NH_4^+-N by different CWM units at three retention times (mean ± SD, n = 24). Different letters as a, b, c and d represent significant variations at $p < 0.05$.

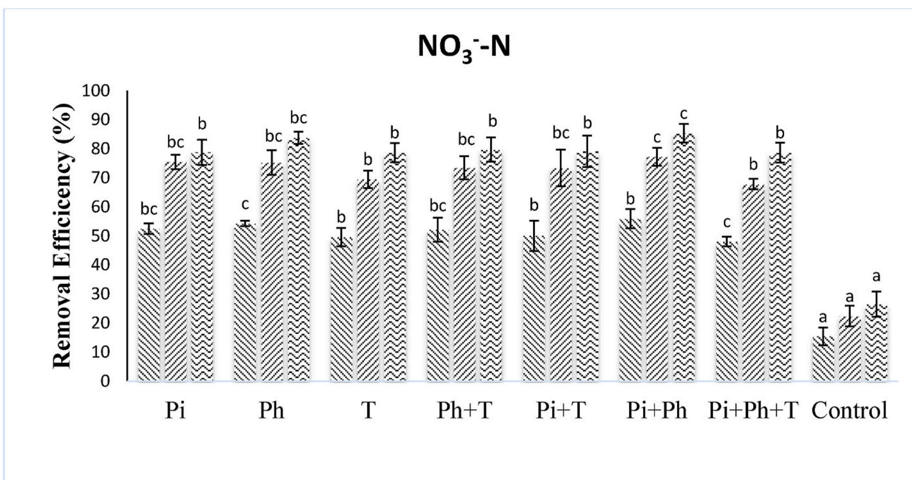

Fig. 6. Removal efficiency of NO_3^--N by different CWM units at three retention times (mean ± SD, n = 24). Different letters as a, b, c and d represent significant variations at $p < 0.05$.

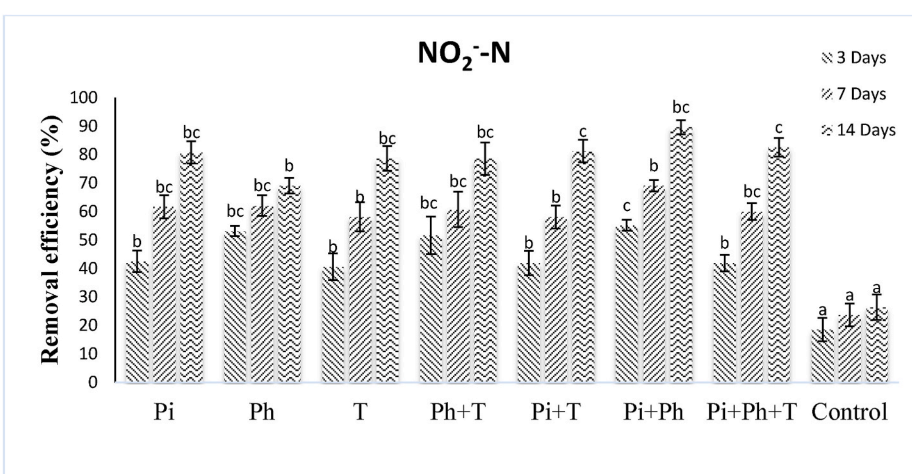

Fig. 7. Removal efficiency of NO_2^--N by different CWM units at three retention times (mean ± SD, n = 24). Different letters as a, b, c and d represent significant variations at $p < 0.05$.

Formal analysis, Validation. **Sampurna Nand:** Formal analysis, Validation. **Bhanu Pratap:** Writing – review & editing. **Divya Dubey:** Visualization, Formal analysis. **Venkatesh Dutta:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2021.129468.

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Interspecific competition and their impacts on the growth of macrophytes and pollutants removal within constructed wetland microcosms treating domestic wastewater

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ABSTRACT

Eight free water surface constructed wetland microcosm (CWM) units are designed with single as well as mixed planting of *Pistia stratiotes, Phragmites karka,* and *Typha latifolia* with control to assess their competitive value (CV), relative growth rates (RGR), and pollutants removal efficiency. Further, the total dry biomass production and other growth parameters such as number of macrophytes, above-ground biomass, below-ground biomass, and root length were also measured to understand the dominant characteristics of the macrophytes. The CWM units with species mixture out-performed species monocultures. Removal of BOD, TP, SRP, NH_4^+-N, NO_3^--N, and NO_2^--N by mixed planting of *P. stratiotes* and *P. karka* was higher at most of the time. *Typha latifolia* was the superior competitor against both *P. stratiotes* and *P. karka* due to its aggressive characteristics that inhibits the growth of neighboring macrophytes. However, *P. karka* was the superior competitor against *P. stratiotes.* The RGR of *T. latifolia* in all experimental units was almost two times more than that of *P. karka.*

Novelty Statement

The CWM units with species mixture out-performed species monocultures. CWMs with more than one macrophytic species are less vulnerable to seasonal fluctuations and more effective in contaminants removal as compared to single macrophyte wetlands. Removal of BOD, TP, SRP, NH_4^+-N, NO_3^--N, and NO_2^--N by mixed planting of *P. stratiotes* and *P. karka* was higher at most of the time. The CWMs with *P. stratiotes* and *P. karka* are superior choice due to their higher wastewater nutrients removal capacity. The application of these three macrophytes in mixed cultures in free water surface constructed wetland is rare. The results are useful in designing large-scale multi-species wetlands which are less susceptible to seasonal variation and more effective in pollutants removal than single-species wetlands.

KEYWORDS
Constructed wetland microcosms; removal efficiency; wastewater pollutants; competitive value; relative growth rate

Introduction

Discharging majority of untreated or partially treated wastewater from communities into nearby water bodies has become a routine problem in developing countries affecting natural ecosystems (Ding *et al.* 2011; Temel Aydı n *et al.* 2018; Kumar and Dutta 2019a). Nitrogen (N) and phosphorus (P) are the key inorganic materials responsible for the eutrophication of water bodies (Spangler *et al.* 2019). Therefore, treatment of domestic wastewater using emergent and free-floating aquatic macrophytes in mixed culture within constructed wetland microcosms (CWMs) provides a viable solution (Kumar and Dutta 2019b). Macrophytes can coexist in the same habitat sharing niche necessities depending on their growth characteristics and dominant behavior (Chesson 2000; Warren *et al.* 2019). Interspecific competition for nutrients, space, and light among different macrophytes are crucial factor in defining wetland vegetation (Connolly *et al.* 2001; Gioria and Osborne 2014; Al-Isawi *et al.* 2015a; Al-Isawi *et al.* 2017; Kankanamge and Kodithuwakku 2017). Uptake of nutrients by aquatic macrophytes in competitive conditions has been of great significance (Zheng *et al.* 2016). CWMs with more than one macrophytic species are vulnerable to seasonal fluctuations and more effective in contaminants removal as compared to single macrophyte wetlands (Liang *et al.* 2011; Bencsik *et al.* 2014; Chang *et al.* 2014). They are known for their cost-effectiveness and environmental friendliness (Wu *et al.* 2013; Al-Isawi *et al.* 2015b; Elfanssi *et al.* 2018; Kumar *et al.* 2020a; Rahi *et al.* 2020), having worldwide application for the treatment of different types of wastewater due to their easy handling and maintenance (Juang and Chen 2007; Svensson *et al.* 2015; Vymazal 2013c; Wu *et al.* 2015). Several abiotic and biotic mechanisms are responsible for efficient

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removal of contaminants, especially rhizospheric region through which wastewater flows (Stottmeister *et al.* 2003).

Macrophytes in CWMs accomplish a number of indirect and direct roles related to the treatment process. The main roles can be identified as assimilation and uptake of wastewater nutrients, providing substrate material, oxygen, and exudates for the growth and attachment of microbial population as well as providing surface insulation, regulation of hydraulics and reduction in wind velocity (Cui *et al.* 2011; Vymazal 2013b; Boog *et al.* 2014; Farzi *et al.* 2017; Hadad *et al.* 2018). In CWMs, emergent macrophytes are capable to assist the rich microbial population growth by developing dense and deep root structure (Jampeetong *et al.* 2012). The cattail (*Typha.* spp.), *Scirpus, Juncus,* and common reed (*Phragmites.* spp.) are the most frequently used species in CWMs (Vymazal 2013a) due to their great reproduction abilities and flood-tolerant capacity (Al-Isawi *et al.* 2016). Both *Typha* spp. and *Phragmites* spp. are colonial macrophytes that share numerous morphological characters, such as tall branched leaves with roots and rhizomes as underground arrangements. They also possess similar habitat and a wide range of ecological settings like resistivity against saline environments (Miklovic and Galatowitsch 2005). Both habitually show dense growth for robust vegetative proliferation (Shih and Finkelstein 2008). The interaction zone among *T. latifolia* and *P. karka* is possibly characterized by strong competition for space and the growth of one species affects neighboring species by spatial dynamics at the interaction zone. Consequently, the neighboring colonies of *T. latifolia* and *P. karka* signify an ideal model about competitive relations and exhibit distinct interspecific competitions in CWMs. However, the application of these two macrophytes in mixed system is rare. *Typha stratiotes* is a free-floating aquatic macrophyte, spreads in the tropics globally, and prefers slow-flowing or static water with high-nutrient availability (Dipu *et al.* 2011). Interspecific competition among macrophytes to acquire nutrients, space and light is one of the most important aspects to determine their growth responses. Nevertheless, due to high inconsistency in competition among several macrophytes, the growth of various macrophytes planted in mixed culture during field scale application is still not clear (Amon *et al.* 2007; Kankanamge and Kodithuwakku 2017). Hence, the objective of this work is to assess the interspecific competition and relative growth rate among macrophytes in mixed culture and their impact on the performance of CWMs. Removal efficiency of wastewater nutrients by particular CWM unit was also studied to define the most appropriate macrophytic combination for its successful operation in future. The results are useful in designing large-scale multi-species wetlands which are less susceptible to seasonal variation and more effective in pollutants removal than single-species wetlands.

Materials and methods

Description of the CWM units

The experiment was conducted in the green house of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (BBAU) Lucknow (26.7697°N, 80.9262°E) UP, India. Free water surface CWM units were setup in concrete containers with dimension $1.2 \times 0.60 \times 0.76$ m (length, width, and depth, respectively) as a batch experiment. All CWM units were filled with $8 \times 8 \times 16$ cm crushed stone, sand, and soil as a substrate material, respectively, as described in previous studies (Shao *et al.* 2014; Kumar *et al.* 2020a). *Pistia stratiotes, Phragmites karka* and *T. latifolia* were planted with initial density of 9 macrophytes per unit as single as well as in combination. An additional set of macrophytes with same weight and height/length were dried at the time of plantation to take their dry weight. The dry weights of macrophytes both at the time of planting and harvesting were used for the analysis of their growth rate and competitive value (Zheng *et al.* 2016; Kankanamge and Kodithuwakku 2017). After the plantation, all the CWM units were filled with tap water and left for one month for macrophytic stabilization (Shao *et al.* 2014; Kumar *et al.* 2020a). The eight planting CWM units on the basis of macrophytes were Pi (*P. stratiotes*), Ph (*P. karka*), T (*T. latifolia*), T + Ph (*T. latifolia* + *P. karka*), T + Pi (*T. latifolia* + *P. stratiotes*), Ph + Pi (*P. karka* + *P. stratiotes*), T + Ph + Pi (*T. latifolia* + *P. karka* + *P. stratiotes*) and an unplanted unit as control (Figure 1). The combination of free-floating and emergent macrophytes were selected on the basis of their pollutants uptake capacity as described previously in several studies (Rezania *et al.* 2019; Kumar *et al.* 2020a; Zhang *et al.* 2021). After the stabilization of macrophytes, domestic wastewater was collected in sterile plastic container from a nearby wastewater drain that received domestic sewage, transported at site and filled in all CWM units equally (Elfanssi *et al.* 2018). Each CWM unit held a total volume of 200 L of untreated domestic wastewater and the hydraulic retention time (HRT) was 3, 7 and 14 d. HRT is the most crucial parameter as it represents time span for which wastewater remains in CWM units. Various studies have been conducted at different HRTs (8 h to 20 d) for the treatment of domestic wastewater (Arias *et al.* 2001; Akratos and Tsihrintzis, 2007; Raphael *et al.* 2019; Kumar *et al.* 2020a). The experiment was conducted in three experimental cycles and each cycle composed of 4 months from March 2018 to February 2019. The characteristic of raw domestic wastewater is given in Table 1. Several environmental factors such as humidity, temperature, and solar intensity were measured on the daily basis. Humidity and temperature were measured by Huger Thermo-hygrometer (8270) and solar intensity through the Luxmeter (LX-101A) provided by HTC™. Analysis of dissolved oxygen (DO) has been done on the daily basis to know the daily variation among different CWM units via Lutron (DO-5509) portable meter. The pH, electrical conductivity (EC), and total dissolved solids (TDS) of wastewater were measured by Hanna portable meter (Hi96107) and HM digital (TDS-3), respectively.

Water sampling and analysis

Effluent samples were collected in 500 mL glass bottles in triplet after 3rd, 7th, and 14th day from each CWM unit.

Figure 1. Eight CWM units designed under the green house of Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow (pictures taken on 18/06/2019 at 4 PM, IST).

All effluent samples were analyzed for ammonium (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), soluble reactive phosphorus (SRP), total phosphorus (TP), and biochemical oxygen demand (BOD) as per the standard methods (APHA 2017). The removal efficiency of several pollutants was calculated using the following equation:

$$\mathrm{RE}(\%) = (1 - C_e/C_i) * 100 \tag{1}$$

where C_i and C_e stand for the influent and effluent concentrations, respectively.

Plant sampling and analysis

The number of individual macrophytes and their height in a particular CWM unit were recorded to evaluate the change in their numbers, total numbers, and height. Macrophytes samples were collected from all CWM units and washed through tap water to remove all debris and separated to measure height and root length. *Pistia stratiotes* produced a number of neonatal plants in addition to increasing the biomass of primary plant. Therefore, measurement for plant height/root length and other growth-related parameters was not taken for *P. stratiotes* (Kankanamge and Kodithuwakku 2017). Macrophytes were separated into above-ground biomass (AGB) and below-ground biomass (BGB) and dried for 48 h in a hot air oven at 103 °C to calculate competitive value (CV). The growth responses among macrophytes grown in mixed culture were evaluated through CV as shown in Equation (2) which offers a mean to illustrate interactions between different macrophytes group (Hong *et al.* 2014):

$$\mathrm{CV} = 100(\mathrm{X}_2 - \mathrm{X}_1)/\mathrm{X}_2 \tag{2}$$

where X_1 is the dry weight of a specific individually grown species and X_2 is the dry weight of a specific species grown with others (mixed planting). However, the relative growth rate (RGR) was evaluated on the basis of formula given by Hunt (1982) and Kankanamge and Kodithuwakku (2017) as provided in the following equation:

$$\mathrm{RGR} = (\log_e \mathrm{W}_2 - \log_e \mathrm{W}_1)/(\mathrm{t}_2 - \mathrm{t}_1) \tag{3}$$

Figure 1. Continued.

Table 1. Characteristics of raw domestic wastewater (DW) (mean ± SD, $n = 12$).

Characteristics of raw domestic wastewater	Biochemical oxygen demand (BOD)	Total phosphorus (TP)	Soluble reactive phosphorus (SRP)	Ammonium (NH_4^+-N)	Nitrate (NO_3^--N)	Nitrite (NO_2^--N)	pH	Electrical conductivity (EC) (mS/cm^2)	Total dissolved solids (TDS)
Concentration (mg L^{-1})	108.11 ± 8.53	11.68 ± 3.15	8.09 ± 1.16	25.56 ± 8.41	12.38 ± 2.76	4.88 ± 2.08	5.49 ± 1.52	0.96 ± 0.15	480 ± 52

where W_1 and W_2 are the average dry weights of macrophytes at the time of plantation (t_1) and harvesting (t_2), respectively.

Statistical analysis

Difference among the mean removal efficiencies and several growth parameters for different CWM units and macrophytes were examined through one-way ANOVA ($p < 0.05$) with post hoc Tukey test for multiple comparison of means. All analyses were performed on SPSS (version 20, SPSS, Chicago, IL), a statistical software package for Social Sciences and Microsoft office XL (version 2016). Data are presented as the mean ± SD.

Results and discussion

Environmental factors

Relative humidity (RH), solar intensity, and temperature (T) were measured to know daily variation in the environmental conditions. The relative humidity ranged from 66% to 90%, solar insolation was within the range of 101 * 100–512 * 100 Lux and temperature ranged from 27 to 36.3 °C. The growth of macrophytes increased significantly in summer months when temperature and relative humidity levels were higher (Licata *et al.* 2019). The higher temperature favors the growth and activity of microorganisms that directly take part in breakdown of several contaminants within the system (Kadlec and Reddy 2001). DO values in several CWM

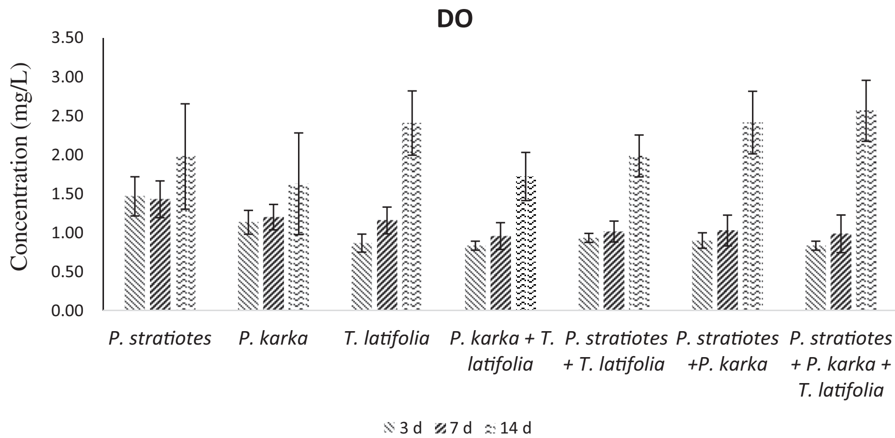

Figure 2. DO concentration among different CWM units at three retention times (mean ± SD).

Table 2. CV and RGR values of macrophytes among different CWM units (mean ± SD, $n = 3$).

	C.V.			RGR (g^{-1} day^{-1})		
Unit	*P. stratiotes*	*P. karka*	*T. latifolia*	*P. karka*	*T. latifolia*	Dominance
P. stratiotes + P. karka	-22.22 ± 3.5^a	38.09 ± 2.32^b	NA	0.018 ± 0.001^b	NA	*P. stratiotes < P. karka*
P. karka + T. latifolia	NA	-30 ± 3.52^a	23.91 ± 2.25^a	0.012 ± 0.002^{ab}	0.02 ± 0.004^a	*P. karka < T. latifolia*
P. stratiotes + T. latifolia	-46.66 ± 2.45^a	NA	50.70 ± 2.8^a	NA	0.026 ± 0.006^b	*P. stratiotes < T. latifolia*
P. stratiotes + P. karka + T. latifolia	-76 ± 4.12^a	-85.71 ± 3.15^a	33.96 ± 3.6^a	0.009 ± 0.001^a	0.023 ± 0.008^a	*P. stratiotes < P. karka < T. latifolia*
P. karka	NA	NA	NA	0.014 ± 0.003^{ab}	NA	NA
T. latifolia	NA	NA	NA	NA	0.022 ± 0.004^a	NA

*NA: not applicable.
Different letters as superscript specify significant differences among means of CV and RGR values of macrophytes planted in several CWM units at $p < 0.05$.

units at different retention times throughout the experiment are shown in Figure 2. The DO varied greatly among various treatment units due to different macrophytic combinations that favors the growth of different microbial communities (Zhang *et al.* 2010, 2021). Initially, the concentration of DO decreased rapidly due to aerobic respiration and chemical oxidation (Ding *et al.* 2011; Xu *et al.* 2021). After that, there was an increasing trend as shown by several CWM units as most of the organic materials were oxidized and taken up by the microorganisms (Ding *et al.* 2011).

Competitive values and relative growth rate among macrophytes

The CV and RGR among the macrophytes were calculated after the completion of 120 day of each experimental period (Table 2). *Pistia stratiotes* showed negative CV with both *T. latifolia* and *P. karka*. Negative CV for *P. stratiotes* with *T. latifolia* and *P. karka* ensured that the total dry biomass of *P. stratiotes* was higher in monoculture than the total biomass in mixed culture. In the same manner, *P. karka* showed negative CV with *T. latifolia* and positive CV with *P. stratiotes*. Negative CV of *P. karka* with *T. latifolia* also implied that the total biomass of *P. karka* was higher in monoculture than the biomass in mixed culture. However, the CV remained positive for *T. latifolia* in all experimental units showing its overall dominance (Zheng *et al.* 2016). Negative CV of *P. stratiotes* with *P. karka* and *T. latifolia* suggests that the interspecific competition with these macrophytes adversely affected the growth of *P. stratiotes* in mixed CWM units (Zheng *et al.* 2016). It is reported that *P. stratiotes* is a weaker competitor in competition with *P. karka*, *T. latifolia*, and *Eichhornia crassipes* (Agami and Reddy 1990; Kankanamge and Kodithuwakku 2017). However, it shows strong competition when planted with *Salvinia auriculata* and *Limnobium laevigatum* and its competitive capability can be improved by supplying additional nutrients (Milne *et al.* 2007). *Typha latifolia* and *P. karka* both are emergent macrophytes; leaves grow upright over the surface of water which restricts the availability of light for free floating *P. stratiotes* (Ali *et al.* 2020). Root biomass per plant is also very small for *P. stratiotes*. Similarly, *P. karka* was the weaker competitor in competition with *T. latifolia* (this study) because *T. latifolia* is considered as dominant and aggressive competitor which restricts the growth of other macrophytes in mixed units with their dense canopy (Kim *et al.* 2018). *Typha latifolia* has the ability to produce more biomass in nutrient rich conditions as compared to others. Consequently, the relative growth rate (RGR) of *T. latifolia* in all experimental units was almost 2 times more than that of *P. karka*. It is reported that the *P.* spp. has been more often used macrophyte for phytoremediation in recent years (Rezania *et al.* 2019). Several studies have been conducted to evaluate competitive behavior between different macrophytes under different ecological situations (Martin and Coetzee 2014). A study carried by Agami and Reddy (1990) in nutrient-enriched wastewater, based on competitive relations between *P. stratiotes* and *E. crassipes* indicated that the *E. crassipes* with dense growth and high plasticity restricted the growth of *P. stratiotes*, by shading and stressing them. *Hydrilla verticillata* was about seven-time superior competitor as compared to *Vallisneria*

Table 3. Number of macrophytes, total number of macrophytes and height/length of macrophytes among all experimental units with respect to time (mean ± SD, $n = 3$).

Days	Unit	No. of Macrophytes			Total No. of Macrophytes	Macrophytes height/ Length (cm)	
		P. stratiotes	*P. karka*	*T. latifolia*		*P. karka*	*T. latifolia*
30 day	*P. stratiotes*	16 ± 3	–	–	16 ± 3	–	–
	P. karka	–	12 ± 2	–	12 ± 2	53.87 ± 10.65[a]	–
	T. latifolia	–	–	15 ± 4	15 ± 4	–	69.61 ± 15.38[a]
	P. stratiotes + *P. karka*	6 ± 2	8 ± 3		14 ± 2	62.52 ± 8.25[b]	–
	P. karka + *T. latifolia*	–	9 ± 2	7 ± 2	16 ± 2	54.81 ± 5.12[a]	69.36 ± 16.02[a]
	P. stratiotes + *T. latifolia*	8 ± 3	–	5 ± 1	13 ± 2	–	83.67 ± 13.2[b]
	P. stratiotes + *P. karka* + *T. latifolia*	4 ± 2	4 ± 1	9 ± 3	17 ± 3	55.93 ± 14[a]	62.15 ± 8.10[a]
60 day	*P. stratiotes*	20 ± 3	–	–	20 ± 3	–	–
	P. karka	–	14 ± 4	–	14 ± 4	68.20 ± 10.52[c]	–
	T. latifolia	–	–	18 ± 3	18 ± 3	–	85.67 ± 6.05[a]
	P. stratiotes + *P. karka*	9 ± 1	12 ± 3	–	21 ± 2	72.54 ± 5.39[c]	–
	P. karka + *T. latifolia*	–	12 ± 4	9 ± 3	21 ± 3	62.23 ± 14.12[b]	92.43 ± 17.25[ab]
	P. stratiotes + *T. latifolia*	12 ± 5	–	10 ± 4	22 ± 5	–	93.98 ± 5.46[b]
	P. stratiotes + *P. karka* + *T. latifolia*	7 ± 4	8 ± 2	14 ± 2	29 ± 4	56.26 ± 11.20[a]	84.56 ± 12[a]
90 day	*P. stratiotes*	31 ± 6	–	–	31 ± 6	–	–
	P. karka	–	19 ± 5	–	19 ± 5	72.54 ± 9.23[bc]	–
	T. latifolia	–	–	21 ± 4	21 ± 4	–	92.50 ± 12.52[ab]
	P. stratiotes + *P. karka*	17 ± 4	16 ± 3	–	33 ± 4	77.84 ± 7.0[c]	–
	P. karka + *T. latifolia*	–	16 ± 5	14 ± 5	30 ± 5	68.54 ± 11.56[ab]	96.82 ± 8.26[bc]
	P. stratiotes + *T. latifolia*	17 ± 7	–	14 ± 4	31 ± 6	–	100.23 ± 8.05[c]
	P. stratiotes + *P. karka* + *T. latifolia*	9 ± 4	12 ± 4	20 ± 5	41 ± 4	63.67 ± 7.14[a]	88.89 ± 9.25[a]
120 day	*P. stratiotes*	28 ± 4	–	–	28 ± 4	–	–
	P. karka	–	24 ± 7	–	24 ± 7	78.23 ± 6.21[bc]	–
	T. latifolia	–	–	21 ± 6	21 ± 6	–	98.15 ± 9.20[a]
	P. stratiotes + *P. karka*	22 ± 7	20 ± 4	–	42 ± 6	83.93 ± 6.15[c]	–
	P. karka + *T. latifolia*	–	22 ± 7	21 ± 6	43 ± 7	73.92 ± 5.12[ab]	101.31 ± 12.25[ab]
	P. stratiotes + *T. latifolia*	25 ± 6	–	18 ± 3	43 ± 5±	–	107.96 ± 7.69[b]
	P. stratiotes + *P. karka* + *T. latifolia*	13 ± 5	15 ± 6	23 ± 4	51 ± 6	65.93 ± 8.15[a]	94.67 ± 10.32[a]

*Different letters as superscript specify significant differences among means of macrophytes length at different time intervals for various CWM units ($p < 0.05$).

americana in excess nutrient conditions (Van *et al.* 1999; Kankanamge and Kodithuwakku 2017). As above, nearly all of the previous findings direct toward the competition among macrophytes, nutrient availability, and environmental conditions, have major effects in determining the competitive capability of a specific species (Mony *et al.* 2007). Several morphological parameters, such as shape of leaf, canopy size, and tall shoot, were related with better competition exhibited by the macrophytes. Occurrence of different macrophytes with neighbors prompts stress on neighboring species for nutrients and space (Craine and Dybzinski 2013). Dissimilar characters enable the competitive ability to acquire necessary resources in settings of higher versus lower resource availability (Martin and Coetzee 2014). It is reported that when the growth of macrophytes is more rapid, the competition for incoming sun light is more significant in nutrient rich conditions. However, in poor nutrient conditions, competition may shift below-ground with growth of roots via nutrient acquisition and growth of macrophytes becomes slow (Gioria and Osborne 2014; Zheng *et al.* 2016). Different macrophytic species could exploit several niches surrounded by the similar ecological conditions, confirming the accessibility to unavailable nutrients for their opponent (Evans and Edwards 2001; Zhang *et al.* 2021). The use of this ability is shown by mixed planting of floating and emergent macrophytes as compared to only floating or emergent macrophytes.

The average height, number of macrophytes, and total number of macrophytes after each 30 day was also recorded in all CWM units by counting number of plants present in particular CWM unit (Table 3). Analysis of variance ($p <$ 0.05) showed that there is a significant difference in plant heights among various single as well as mixed experimental units. The maximum height of *T. latifolia* was observed in unit Pi + T and *P. karka* in unit Pi + Ph, both in combination with *P. stratiotes* in all four samplings.

Growth characteristics such as total biomass, AGB, BGB, and root length (RL) of *P. karka* and *T. latifolia* was measured at the end of experimental period. One-way ANNOVA analysis ($p = 0.05$) to compare means of several growth parameters for different treatment units showed that there is a significant difference among means of several growth parameters with respect to treatment units. The total biomass, AGB, and BGB (g/plant) of *P. karka* were found to be higher in unit Pi + Ph whereas RL (cm) was maximum in unit Ph. However, for *T. latifolia*, the maximum values of total biomass, AGB, and BGB were shown by Pi + T and maximum RL by T as compared to other units (Table 4).

Nutrient removal efficiency of CWMs

Average removal efficiencies for BOD, TP, SRP, NH_4^+-N, NO_3^--N, and NO_2^--N among different CWM units designed using emergent and free-floating macrophytes in a single as well as in mixed planting with respect to different retention times have been shown in Figure 3(A–F). Analysis of variance ($p < 0.05$) among mean removal efficiencies for different contaminants within various CWM units showed there is significant difference among removal efficiencies with respect to different CWM units and retention times. Maximum removal of BOD was exhibited by CWM unit Ph + Pi (62.58%, 86.14%, and 92.34% for 3, 7, and 14 days,

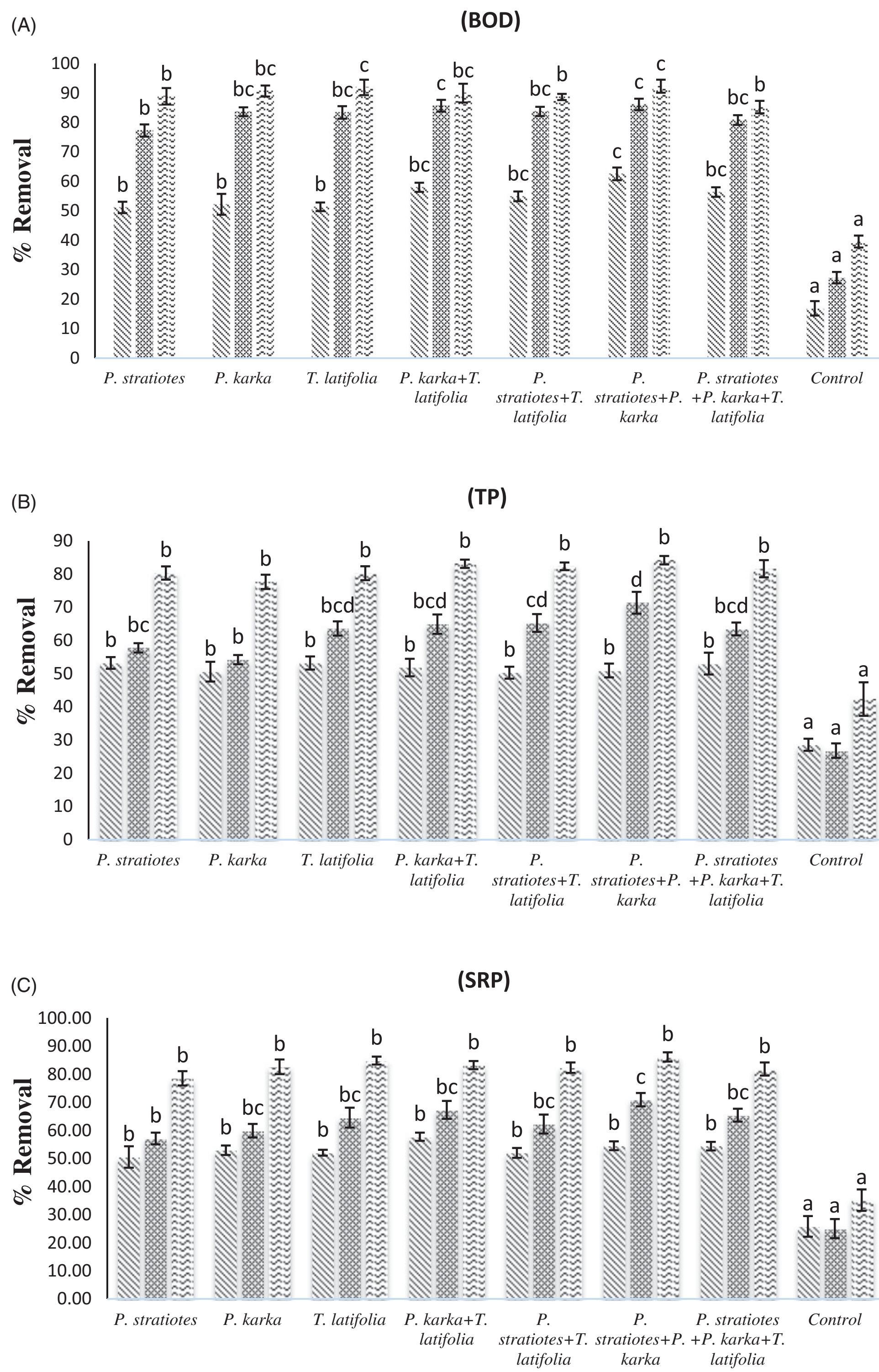

Figure 3. (A, B, C, D, E, and F) Average removal efficiencies for BOD, TP, SRP, NH_4^+-N, NO_3^--N, and NO_2^--N from different CWM units at three retention times respectively (mean ± SD, $n = 12$). Different letters (a, b, c and d) above the error bars represent significant differences among the mean contaminant removal efficiencies at different HRTs for various CWM units at $p < 0.05$.

respectively) (Figure 3(A)). Similar results were observed in previous studies (Kumar *et al.* 2020a). The removal efficiency for TP ranged from 50% to 53%, 54.19% to 71.32%, and 77.62% to 84.14% among different treatment units for 3, 7, and 14 days, respectively. The maximum removal capacity exhibited by CWM units Pi (53.20%), Ph + Pi (71.32%), and again by CWM unit Ph + Pi (84.14%) at 3, 7, and 14 days, respectively (Figure 3(B)). It is observed that the free water surface wetlands with emergent and free-floating macrophytes are more appropriate toward the removal of nutrients especially phosphorus (Zheng *et al.* 2016). This is due to the influence of macrophytic uptake, substrates adsorption, and microbial degradation, which are quite dissimilar in free water surface CWMs as compared to

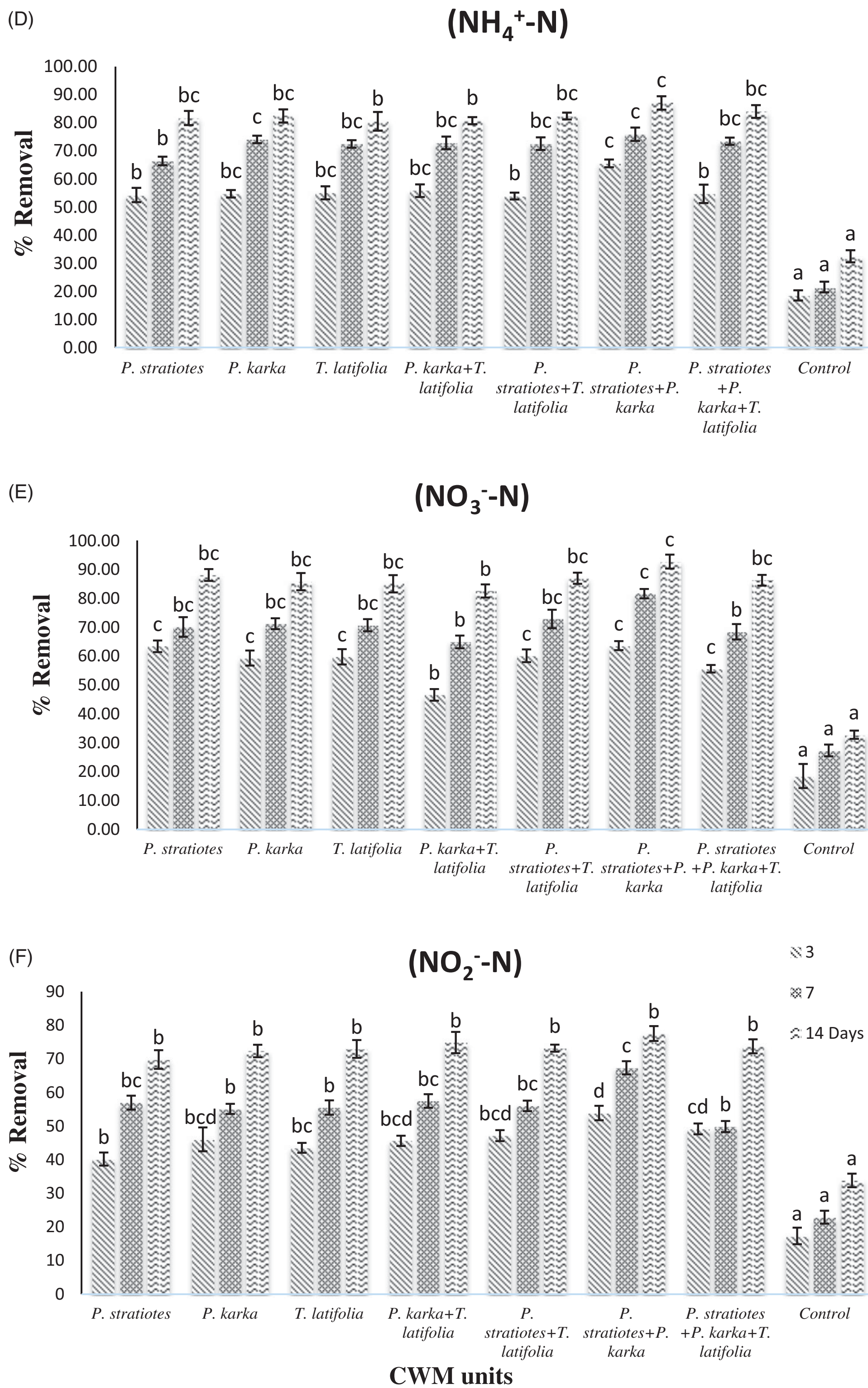

Figure 3. Continued.

subsurface flow wetlands (Kadlec and Wallace 2008). The rest of experimental units also performed well as described in previous research (Kankanamge and Kodithuwakku 2017; Zhai *et al.* 2016; Hickey *et al.* 2018). It is reported by Yasar *et al.* (2018) that the *P. stratiotes* and *P. karka* are more efficient toward removal of phosphate that showed up to 90% and 73% removal efficiency, respectively. They also state that the *P. stratiotes* was known for its higher removal efficiency toward physical as well as chemical contaminants. Similarly, maximum removal efficiency for SRP was exhibited by CWM units T + Ph (57.73%) for 3 days retention time and Ph + Pi (70.94% and 86.15%) for 7 and 14 days, respectively (Figure 3(C)). Removal efficiency for NH_4^+-N was found higher in the CWM unit Ph + Pi (65.54%, 75.89%, and 87.02% for 3, 7, and 14 days, respectively) planted with *P. stratiotes* and *P. karka* at all three retention

Table 4. Growth characteristics of macrophytes at the end of experiment (mean ± SD, $n = 3$).

Units	Total biomass (g/plant)	AGB (g plant^{-1})	BGB (g plant^{-1})	Root length (cm plant^{-1})
P. stratiotes	5 ± 1^{a}	4.3 ± 0.95^{ab}	0.7 ± 0.02^{a}	5 ± 2.1^{ab}
P. karka	24.63 ± 3.65^{a}	11.33 ± 2.5^{a}	13.3 ± 0.8	$28. \pm 2.08^{c}$
T. latifolia	59.72 ± 4.89^{a}	45.33 ± 3^{a}	14.39 ± 2.0^{ab}	$41.22.66 \pm 0.57^{b}$
P. stratiotes + *P. karka*	$(4.5 \pm 0.8^{a}) + (24.95 \pm 8.12^{b})$	$(3 \pm 1.2^{a}) + (12.67 \pm 1.2^{b})$	$(1.5 \pm 0.5^{a}) + (12.28 \pm 1.32^{b})$	$(7 \pm 2.5^{b}) + (22.20 \pm 1.63^{bc})$
P. karka + *T. latifolia*	$(23.44 \pm 3.32^{a}) + (68.76 \pm 7.21^{a})$	$(10.33 \pm 1.8^{a}) + (47.33 \pm 1.6^{a})$	$(13.13 \pm 0.9^{a}) + (21.43 \pm 3.1^{a})$	$(19.95 \pm 1.3^{a}) + (39.12 \pm 10.44^{b})$
P. stratiotes + *T. latifolia*	$(4 \pm 0.91^{a}) + (87.91 \pm 12.42^{b})$	$(3 \pm 0.7^{a}) + (74.33 \pm 3.2^{c})$	$(1 \pm 0.05^{a}) + (13.58 \pm 0.7^{ab})$	$(4.6 \pm 2^{a}) + (33.13 \pm 0.5^{a})$
P. stratiotes + *P. karka* + *T. latifolia*	$(3 \pm 1.2^{b}) + (17.2 \pm 2.15^{a}) + (82.18 \pm 6.31^{b})$	$(2.4 \pm 1^{a}) + (8.67 \pm 1.1^{a}) + (65.33 \pm 2.3^{b})$	$(0.6 \pm 0.002^{a}) + (8.53 \pm 0.6^{a}) + (16.85 \pm 4.5^{b})$	$(4 \pm 1^{b}) + (21.96 \pm 0.8^{ab}) + (36.53 \pm 0.57^{ab})$

*Different letters as superscript specify significant differences among means of macrophytes weights and root length planted in CWM units at $p < 0.05$.

times (Figure 3(D)). Similar results were found in several other studies for municipal wastewater treatment (Zhai *et al.* 2016: Hickey *et al.* 2018). Maximum removal capability for NO_3^--N was exhibited by CWM unit Ph + Pi (63.66%, 81.66%, and 92.77%) at 3, 7, and 14 days, respectively, as observed in our previous study also (Kumar *et al.* 2020a) (Figure 3(E)). Jampeetong *et al.* (2012) proposed that the rate of N_2 uptake is linked with the growth rate of macrophytes. It does not mean that macrophytes with higher RGR or superior competitor always show higher removal efficiency toward nutrients. Thus, this study does not support the recommendations given by Jampeetong *et al.* (2012). Therefore, the maximum nutrient uptake capability may be a characteristic of the macrophyte and the capacity to accumulate more nutrients in their tissues based on the availability as shown by *P. stratiotes* and *P. karka* (Tanner 1996; Zhang *et al.* 2021). It is reported that *P. stratiotes* is able to take nutrients directly from the CWMs (Jampeetong *et al.* 2012). The maximum removal efficiency for NO_2^--N was shown again by the CWM unit Ph + Pi (53.90%, 67.34%, and 77.65%) for 3, 7, and 14 days of retention times respectively as compared to other single as well as mixed CWM units (Figure 3(F)). The other CWM units also showed significant removal performances for all selected wastewater pollutants throughout the experiment.

Removal of wastewater nutrients through uptake by macrophytes in CWMs with free-floating and emergent macrophytes is a most significant mechanism as compared to CWMs with emergent macrophytes only (Vymazal 2007; Kumar *et al.* 2020b). In this study, unit planted with *P. stratiotes* and *P. karka* (Pi + Ph) showed maximum removal efficiency for all selected parameters at most of the time as compared to other mixed as well as single planting units. This is due to their high nutrient uptake capacity, providing more oxygen to aerobic bacteria, presence of diverse microorganisms, regenerating power, vigorous growth, and less susceptibility toward seasonal variations (Leto *et al.* 2013; Zheng *et al.* 2016; Rahi *et al.* 2020). Several other CWM units also performed well for the removal of BOD and nutrients. The removal efficiency can be enhanced further by intermittent aeration (Liu *et al.* 2019). The evaluation of nutrients concentration in tissues of macrophytes in plants per gram dry weights was less significant concerning nutrient removal capacity, as it differs greatly by the quantity of biomass (Zheng *et al.* 2016). It is reported that the increased macrophytic biomass, and densities in mixed units as well as the enzyme activities within the substrate material, enhanced nitrogen removal efficacy (Leto et al. 2013; Sun *et al.* 2019). The roots of macrophytes may help in fostering a rich bacterial growth as biofilms which enhance the removal of nutrients (Zheng *et al.* 2016). Toscano *et al.* (2015) evaluated that the richness of bacteria and the removal efficiency of CWMs differs significantly in relation to dissimilar macrophytic species. Therefore, they recommended the use of mixed planting systems to enhance the performance of wastewater treatment. CWMs with mixed planting were also known for their less susceptibility toward seasonal variations (Liang *et al.* 2011; Chang *et al.* 2014). However, the applications of these mixed planting CWMs were limited to field scale operations (Zhang *et al*. 2021).

Conclusion

Interspecific competition and nutrient removal efficiency of different surface flow constructed wetland microcosms were investigated with single as well as the mixed culture of macrophytes. There is notable effect of interspecific competition between macrophytes in CWM units developed for the treatment of domestic wastewater. *Pistia stratiotes* is a weaker competitor when competing with *P. karka* and *T. latifolia.* Negative CV values of *P. stratiotes* with *P. karka* and *T. latifolia* imply that the competition adversely affected the growth and biomass in mixed units as compared to single planting units. *Phragmites karka* was a superior competitor with *P. stratiotes* and weaker competitor with *T. latifolia. Typha latifolia* was always found superior competitor among all macrophytes. Superior competitors gain relatively higher biomass due to their dominant character to occupy more space and nutrients. The relative growth rate of *T. latifolia* in all experimental units was always two times more than that of *P. karka.* The interaction zone among *T. latifolia* and *P. karka* is possibly characterized by strong competition for space and the growth of one species affecting neighboring species by spatial dynamics at the interaction zone. Consequently, the neighboring colonies of *T. latifolia* and *P. karka* signify an ideal model about competitive relations and exhibit distinct interspecific competitions. Removal of wastewater nutrients among all CWM units varied greatly. Removal of nutrients via uptake by free-floating and emergent macrophytes is a most significant mechanism as compared to emergent macrophytes within a CWM. Removal of BOD, NH_4^+-N, NO_3^--N, NO_2^--N, TP and SRP by mixed planting of *P. stratiotes* and *P. karka* was higher at most of the time as compared to other mixed as well as single planting units due to their high nutrient uptake capacity, providing more oxygen to aerobic bacteria, presence of diverse microorganisms, regenerating power, vigorous growth, and less susceptibility toward seasonal variations. From the above findings, it is observed that interspecific competition causes different growth responses of plant species including competitive interactions for space between macrophytes vis-à-vis the treatment performance. The results are useful in designing large-scale multi-species wetlands which are less susceptible to seasonal variation and more effective in pollutants removal than single-species wetlands.

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Variation in extracellular enzyme activities and their influence on the performance of surface-flow constructed wetland microcosms (CWMs)

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HIGHLIGHTS

- Significant difference in enzyme activities observed with respect to time of the year.
- Top layer of CWM units exhibits significantly higher enzyme activities.
- Unit with *Phragmites karka* and *Pistia stratiotes* shows maximum enzyme activities.
- Most of the CWM units show significant positive correlation between pollutants removal and enzyme activities.

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ABSTRACT

Eight constructed wetland microcosm (CWM) units have been designed using three macrophytes for domestic wastewater treatment. The main aim of this study is to evaluate enzyme activities with respect to time and soil depth and their correlation with removal efficiency of pollutants within different CWM units. The findings of this study show that the activity of enzymes and pollutants removal efficiency vary to a great extent on the soil depth, time of the sampling and type of pollutants. The correlation between removal of soluble reactive phosphorus and total phosphorus was significant with phosphatase activity in most of the CWM units. Activity of urease and NH_4^+-N removal was positively correlated with significant positive correlation in CWM units planted with *Phragmites karka*, and *Pistia stratiotes* (*Ph* + *Pi*) and *Typha latifolia*, *Phragmites karka* and *Pistia stratiotes* (*T* + *Ph* + *Pi*). Urease activity was found to be both positively and negatively correlated with respect to removal of NO_3^--N and NO_2^--N in different CWM units. Dehydrogenase activity showed negative correlation with respect to biological oxygen demand (BOD) removal except in CWM units with *Ph* + *Pi* and *T* + *Ph* + *Pi*. Similarly, a moderate positive and negative correlation exists between fluorescein diacetate hydrolysis and BOD removal. Removal of BOD and microbial biomass carbon (MBC) was negatively correlated with each other in most of the CWM units. With respect to vertical variation, the top layer of CWM units expressed significantly higher activity of extracellular enzymes and were significantly different from the deeper layer. CWM units exhibited significant variations in enzyme activity with respect to time.

1. Introduction

Wastewater treatment using CWMs implies the conversion of complex organic contaminants into simpler compounds and inorganic substances (Kong et al., 2009). This is accomplished through the activity of enzymes (Martens et al., 1992; Kang et al., 1998) and microbial metabolism within soil substrates (Brix and Schierup, 1989; Chen et al., 2020). Activity of enzymes is suggested as a significant determining factor to enhance water quality in CWMs (Freeman et al., 1997; Shackle et al., 2000; Yan et al., 2018). The activity of enzymes within CWMs is influenced by several factors such as biotic features (macrophytic diversity, population of microorganisms, fauna and higher taxa), edaphic factors (pH, organics and nutrient availability, depth and soil texture) and prevailing

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climatic conditions (Zaman et al., 1999; Duarte et al., 2008; Reboreda and Caçador, 2008). The activity of enzymes could also be altered by influencing the quality and quantity of carbon source to enhance the performance of CWMs (Shackle et al., 2000). The supply of exogenous enzymes improves the biodegradation processes within the CWMs (Shackle et al., 2006). The enzymes that are deactivated by certain chemical compounds such as tannins are reactivated by root system of the macrophytes through oxygenation (Neori et al., 2000). The activity of active enzymes is maximum nearby the rhizosphere (Zhang et al., 2007). There are 12 soil enzymes evaluated by Niemi et al. (2005) that exhibit positive association among root biomass of macrophytes and activity of enzymes. Macrophytes are able to alter the enzyme activity via emitting extracellular enzymes. Macrophytes can also influence community structure and variety of microorganisms through liberating oxygen and root exudates into the rhizospheric region that have direct effect on the activity of enzyme. A positive connection among enzyme activity and root biomass of macrophytes within the rhizosphere was also reported (Reboreda and Caçador, 2008). The root biomass of several macrophytes have significant effect on activity of enzymes within the CWMs. Various earlier studies reported that the biomass of fine roots have strong correlation with removal efficiency as compared to biomass of total root in a CWM system. (Yang et al., 2007; Cheng et al., 2009). Therefore, the activity and growth status of roots may be crucial than the total biomass of roots to influence enzymes activity. CWMs are artificially engineered ecosystems that use natural drivers to treat several types of wastewater. Basically, there are three components of CWMs namely macrophytes, supportive substrate material and associated microbial assemblage (Kumar and Dutta, 2019a). Together they utilize natural processes to remove or transform wastewater contaminants. Along with contaminant removal, they also provide several environmental benefits such as groundwater recharge, flood control, provide habitat for valuable wildlife, aquaculture, fisheries, carbon sequestration, add aesthetic values and recreational uses (Liang et al., 2017; Kumar and Dutta, 2019b). The aim of this study is to evaluate the activity of different extracellular soil enzymes in CWM units planted with different macrophytic combinations and their relation with contaminant removal efficiency.

2. Material and methods

2.1. Description of site

The experimental setup was designed in green house at Babasaheb Bhimrao Ambedkar University, Lucknow (26.7697° N, 80.9262° E), India. CWM units were developed using crushed stone (Diameter 2–4 cm), sand and soil as a substrate media in 8-concrete containers (dimension L * W *D = 1.2 * 0.60 * 0.76 m) as a batch experiment. The uppermost zone (16 cm) was filled with soil collected from nearby water logged area within the university campus; middle zone contained 8 cm layer of washed river sand and the bottom zone contained 8 cm of crushed stone (diameter 2–4 cm). *Phragmites karka, Typha latifolia* and *Pistia stratiotes* that had almost same weight/length were planted with initial density of 18 macrophytes per unit as single as well as in combination. Selection of macrophytes is based on their contaminant uptake capacity as reported in the literature. These macrophytes are frequently used in CWs for the treatment of different types of wastewater in several continents such as Asia and Europe. The CWM units were then left for acclimatization for about one month after filling through tap water. CWM units with different plant combinations have been given in Fig. 1. Domestic wastewater collected from a nearby wastewater stream was transported in a 200-L plastic container and filled in all CWM units. Each treatment unit held a total volume of 200 L of wastewater. The HRT (hydraulic retention time) was 3, 7 and 14 days and the experiments were carried for one year. The annual average temperature ranged from 25.70 to 31.33 ^{O}C, humidity 66–90% and solar intensity 101 × 100–512 × 100 Lux. Average dissolved oxygen (DO) and pH of the domestic wastewater utilized in present study during the experiment was 1.20 ± 0.52 and 5.48 ± 0.52 mg L^{-1} respectively. Characteristics of raw domestic wastewater utilized in this study has been given in Table 1.

2.2. Sample collection and analysis

Effluent samples from each CWM units were collected in 500 mL glass bottles in triplet after 3, 7 and 14 d. The experiment was done for one year during October 2018 to September 2019 and analyzed for BOD, TP, SRP, NO_3^--N and NO_2^--N as per the guidelines given by Apha and WEF (2012). The samples of soil from both top and deeper layer (0–10 and 10–15 cm) were collected every month from all CWM units during the experimental cycles. To remove plant parts and other materials present, soil samples were screened using mesh (1 mm) and then assayed for the activity of dehydrogenase (DHA), phosphatase, urease, microbial biomass carbon (MBC) and fluorescein diacetate (FDA) hydrolysis.

2.3. Enzyme activity assay

DHA activity was evaluated using reduction technique (modified 2,3,5- TTC (triphenyl tetrazolium chloride) provided by Małachowska-Jutsz and Matyja (2019). 5 g of soil sample was weighed and placed in culture tubes followed by mixing it with $CaCO_3$ (0.1 g) and distilled water (1.5 mL). Then, 1 mL of 1% TTC reagent was added. All the tubes were plugged with cotton and transferred in an orbital incubator for 24 h at 30 °C. The solution was then transferred on filter paper and TPF (triphenyl formazan) was taken out sequentially using methanol in a flask. The appearance of the pink color was read out via UV–Visible spectrophotometer (Systronics - 2203) using methanol as control at 485 nm.

Activity of phosphatase was quantified as follows; soil sample (1 g) was mixed with toluene solution (0.25 mL), and 4 mL of acetate buffer (pH 5.8). After that, 0.25 mL of substrate solution and 0.115 M pNPP (p-nitrophenol phosphate) was mixed and placed in an orbital incubator at 37 °C for 1 h. After incubation, the reaction was terminated by addition of 0.5 M NaOH (4 mL). In this reaction, development of p-nitrophenol was evaluated by UV–Visible spectrophotometer (Systronics- 2203) at wavelength 400 nm. The activity of phosphatase enzyme within soil samples was stated as μg p-nitrophenol g^{-1} soil h^{-1} (Schinner and Von Mersi, 1990).

The activity of urease was measured by procedure given by Klose and Tabatabai (2000). 5 g of soil sample was taken and then 10 mL of phosphate buffer solution (pH 6.7) was added, followed by addition of toluene (0.5 mL) and 10% solution of urea (10 mL). All the ingredients were mixed and incubated at 37 °C for 48 h. After the incubation, 1 M KCl solution (20 mL) was added and all the contents were thoroughly mixed for 30 min and filtered out. 1 mL of filtrate was mixed with 9 mL of deionized water to make final volume of 10 mL. This was followed by addition of potassium sodium tartrate (1 mL) and Nessler reagent (0.8 mL). In the same solution, 4 mL 1 M NaOH was added and the sample was adjusted to 25 mL using deionized water. The release of NH_4 was read out spectrophotometrically (Systronics - 2203) at 460 nm. The activity

Fig. 1. Eight CWM units showing emergent and free-floating macrophytes in single as well as mixed planting in the green house. Here, *Pi* stands for *Pistia stratiotes*, *Ph* for *Phragmites karka*, and *T* for *Typha latifolia* and control as unplanted unit. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Average concentration of contaminants in the low to medium strength domestic wastewater fed into CWMs (mean ± SD).

Characteristics of raw domestic wastewater	Total phosphorus (TP)	Soluble Reactive Phosphorus (SRP)	Biological Oxygen Demand (BOD)	Ammonium (NH_4^+-N)	Nitrate (NO_3^--N)	Nitrite (NO_2^--N)
Concentration (mgL^{-1})	11.67 ± 2.85	8.07 ± 2.60	108.11 ± 11.69	25.56 ± 6.26	12.38 ± 4.56	4.88 ± 2.08

of urease in the soil samples was shown as μg NH_4 g^{-1} soil 24 h^{-1}.

Estimation of MBC within soil samples followed chloroform-fumigation method as prescribed by Anderson and Ingram (1993). Initially, to remove plant materials and stones, the samples were sieved via mesh having pore size of 1 mm. After sieving, 10 g of soil sample was taken in a 250 mL conical flask for fumigated extraction (ct1) in replicates of three. The conical flask containing soil samples and 30 mL chloroform were then sited in a vacuum desiccator and vacuum was used until the chloroform evaporated completely. After that, the desiccator was located in the light deficient region for 5 day at 25 °C. Similarly, for unfumigated extraction (ct2); the soil sample (10 g) was sited in a water tight extraction bottle (125 mL) and extracted directly using 0.5 M K_2SO_4 (50 mL) with gently mixing for 30 min. The fumigated soil samples were also taken out like the unfumigated soil samples after five days. The sample extracts were then filtered out and 4 mL of filtrate was pipetted out in a conical flask. 1 mL of potassium dichromate (0.0667 M) and 5 mL of 98% H_2SO_4 were added and the flask was placed on hot plate to heat for 30 min at 150 °C. The digested samples were transferred in another flask and 0.3 mL of phenanthroline monohydrate was added as an indicator. All the samples were then titrated using FAS (ferrous ammonium sulphate) solution and the change in color from green to violet red was evaluated as an end point. MBC was expressed in μg g^{-1} of soil sample.

Estimation of FDA hydrolysis was done using method described by Green et al. (2006). The soil sample (1 g) was sited in a flask and then 15 mL of potassium phosphate buffer and FDA solution (0.2 mL) was added. The flasks containing all these reagents were put in an orbital incubator for 3 h at 24 ^{O}C, which was followed by addition of 15 mL of extracting solution (methanol/chloroform as 1:2 v/v). The content was then centrifuged at 10,000 rpm for 3 min. The supernatant was filtered and the intensity of FDA hydrolysis was measured using UV–Visible spectrophotometer (Systronics - 2203) at 490 nm. The hydrolysis of FDA hydrolysis was stated as μg g^{-1} h^{-1}.

2.4. Statistical analysis

Analysis of the experimental data presented here has been done using Statistical analysis tool SPSS (version 20.0) and MS office Excel (version 2016). One-way annova ($p < 0.05$) was performed for the analysis of variance to compare the means of enzyme activity within different CWM units with respect to retention time and soil depth. Pearson correlation coefficient was calculated to evaluate the correlation between pollutant removal efficiencies and enzyme activities.

3. Results

3.1. Vertical variation in activity of extracellular enzymes

Top layer (0–10 cm) of CWM units expressed significantly higher activity of extracellular enzymes ($p < 0.05$) in all of the CWM units and differed strongly from deeper layers (10–15 cm) (Fig. 2). Significant differences in enzymes activity were observed in the different CWM units with different combination of macrophytes. CWM unit planted with *Phragmites karka* and *Pistia stratiotes* showed higher values of enzymes activity in the top as well in deeper layer for most of the enzymes except MBC. Higher MBC was shown by the unit planted with *Typha latifolia.* Higher urease, FDA and phosphatase activities were measured in unit planted with *Phragmites karka* and *Pistia stratiotes* (*Ph* + *Pi*). Several CWM units planted with *Typha latifolia* + *Phragmites karka* (*T* + *Ph*) and *Phragmites karka* (*Ph*) also performed well towards activity of several enzymes at both layers of soil samples.

3.2. Temporal variation in activity of extracellular enzymes

The activity of DHA, urease, phosphatase, FDA and MBC exhibited significant variations over time. The temporal variation in all enzyme's activity showed almost similar pattern among the all the treatment units. In the uppermost layer of soil samples (0–10 cm), highest DHA was observed in June for CWM unit with *Ph* + *Pi* and for the deeper layer (10–15 cm), the highest activity was exhibited by unit with *Ph* and *T* + *Ph* in April and July respectively (Fig. 3). Several other units at different time periods also exhibited nearly similar DHA. The control unit represented higher DHA also in June and October for top as well as deeper layer of soil samples respectively. Maximum urease activity for upper soil layer was observed in April for the CWM unit with *Ph* + *Pi* and for deeper layer, maximum urease activity was exhibited by CWM unit with *Ph* in March (Fig. 4). Several other CWM units also showed nearly equal urease activity in October, May and June. The highest urease activity in control unit for top and deeper both soil layers was observed in May and June respectively. Highest phosphatase activity for upper and deeper layer of soil sample was shown by CWM unit with *Ph* + *Pi* in May and October respectively (Fig. 5). However, the control unit showed highest phosphatase activity in April for both layers of soil samples. Similarly, highest activity of FDA hydrolysis for top layer of soil sample was exhibited by CWM unit with *Ph* + *Pi* in June (Fig. 6). In the deeper layer of soil sample, the variation is less distinctive; with higher activity in June for CWM unit with *T.* The control unit showed higher FDA hydrolysis in October and May for top and deeper layer of soil sample. In the top foremost layer of soil samples, MBC showed a multi-peaks pattern

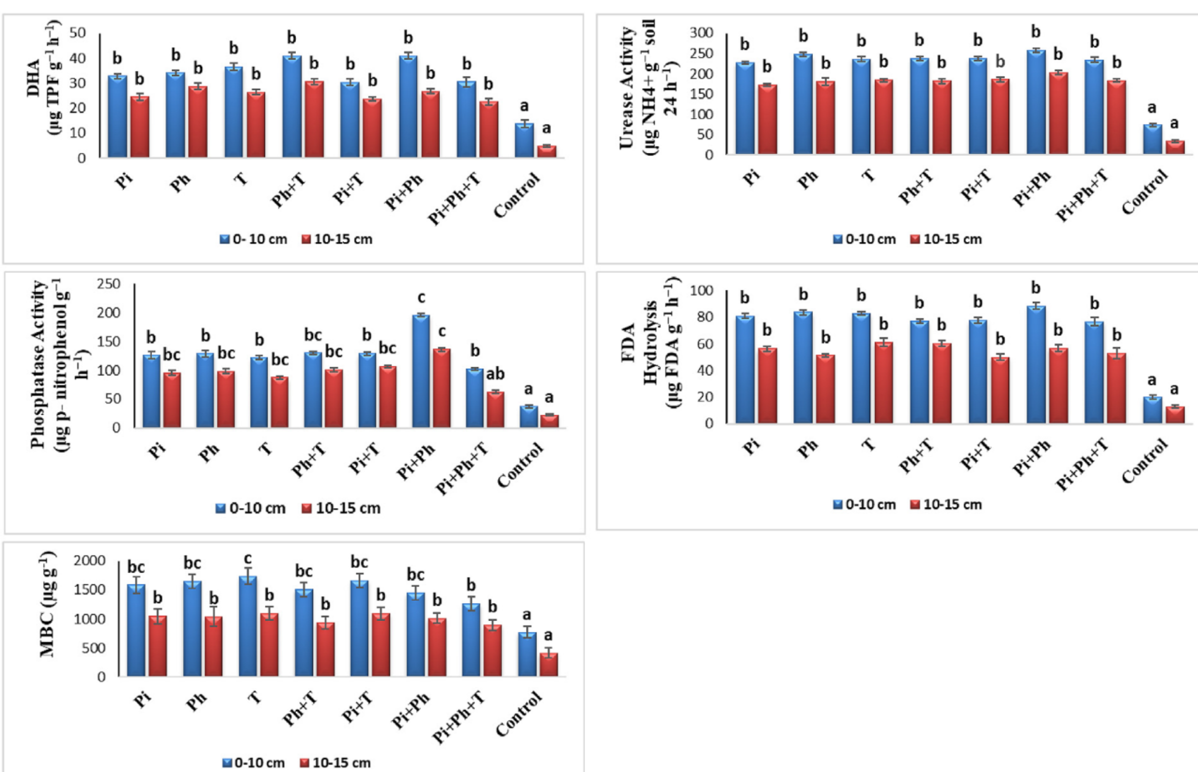

Fig. 2. Vertical variation in extracellular enzymes activity at two consecutive soil layers in the macrophyte-specific CWM units (mean ± SD). Different letters as superscript specify significant differences among means of enzyme activities within CWM units at $p < 0.05$.

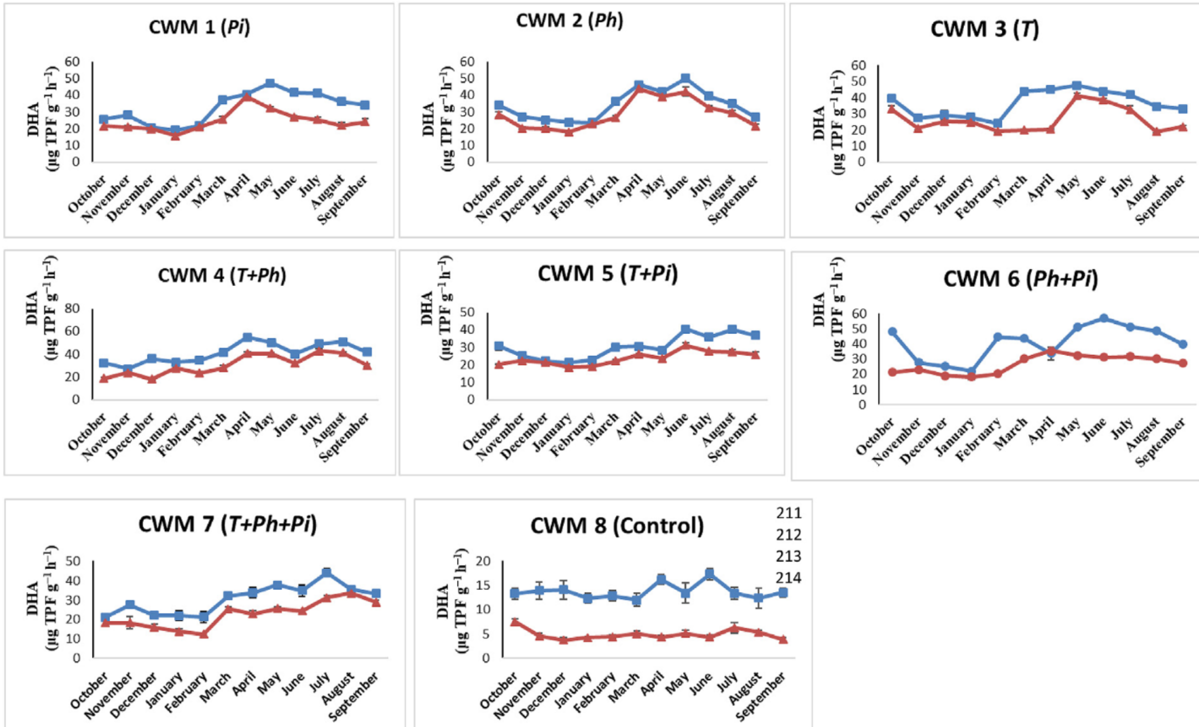

Fig. 3. Temporal variation in DHA at two consecutive soil layers in the macrophyte-specific CWM units (mean ± SD).

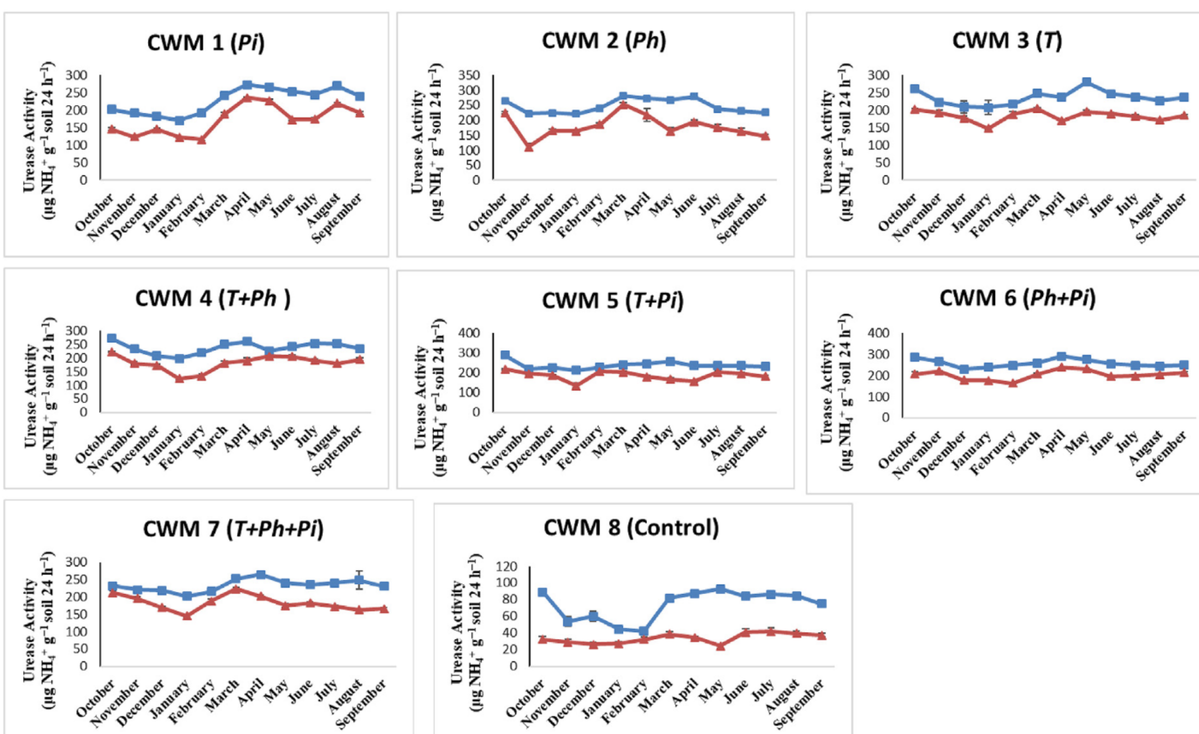

Fig. 4. Temporal variation in urease activity at two consecutive soil layers in the macrophyte-specific CWM units (mean ± SD).

with higher values in March and May for bottom layer of soil samples in CWM unit with *T* (Fig. 7). However, the control unit exhibited highest MBC in March for both layers of soil samples.

3.3. *Relationship among activity of enzymes and pollutant removal efficacy*

Strong correlation exists among activity of phosphatase and TP

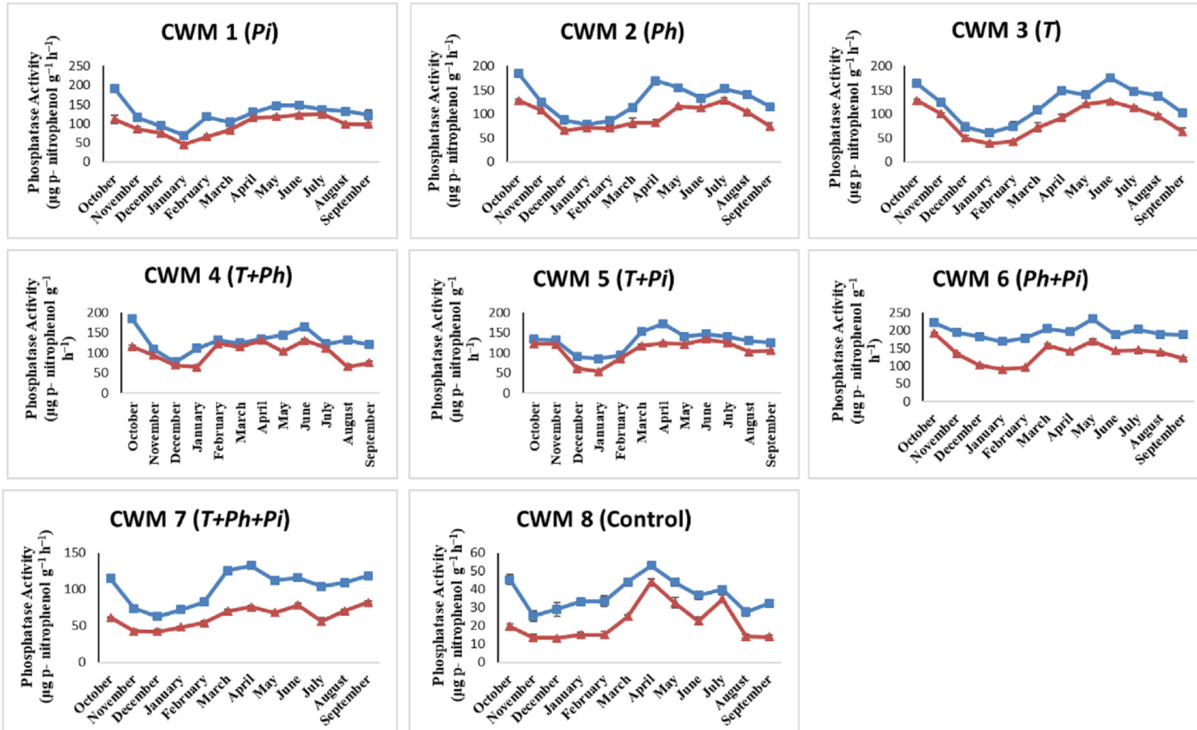

Fig. 5. Temporal variation in phosphatase activity at two consecutive soil layers in the macrophyte-specific CWM units (mean ± SD).

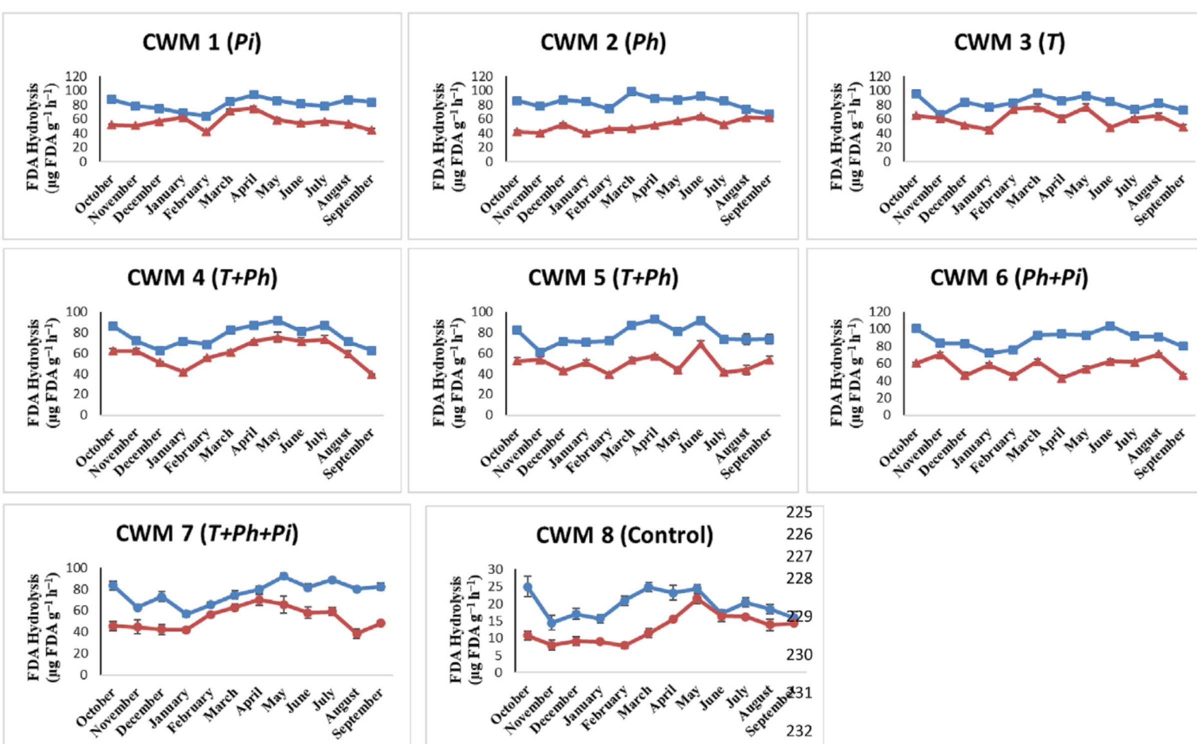

Fig. 6. Temporal variation in FDA hydrolysis at two consecutive soil layers in the macrophyte-specific CWM units (mean ± SD).

removal. Majority of CWM units have significant positive correlation between TP removal efficiencies (at 3, 7- and 14-d retention time) and phosphatase activity at both depths (Table 2). Phosphatase activity with SRP removal efficiencies have positive correlation in CWM units with *Pi*, *Ph* and *Ph* + *Pi* and significant negative correlation in units with *T* + *Ph*, *T* + *Pi* and *T* + *Ph* + *Pi*. Activity of urease and NH_4^+-N removal showed positive correlation in most of the CWM units with significant correlation in CWM units with *Ph* +

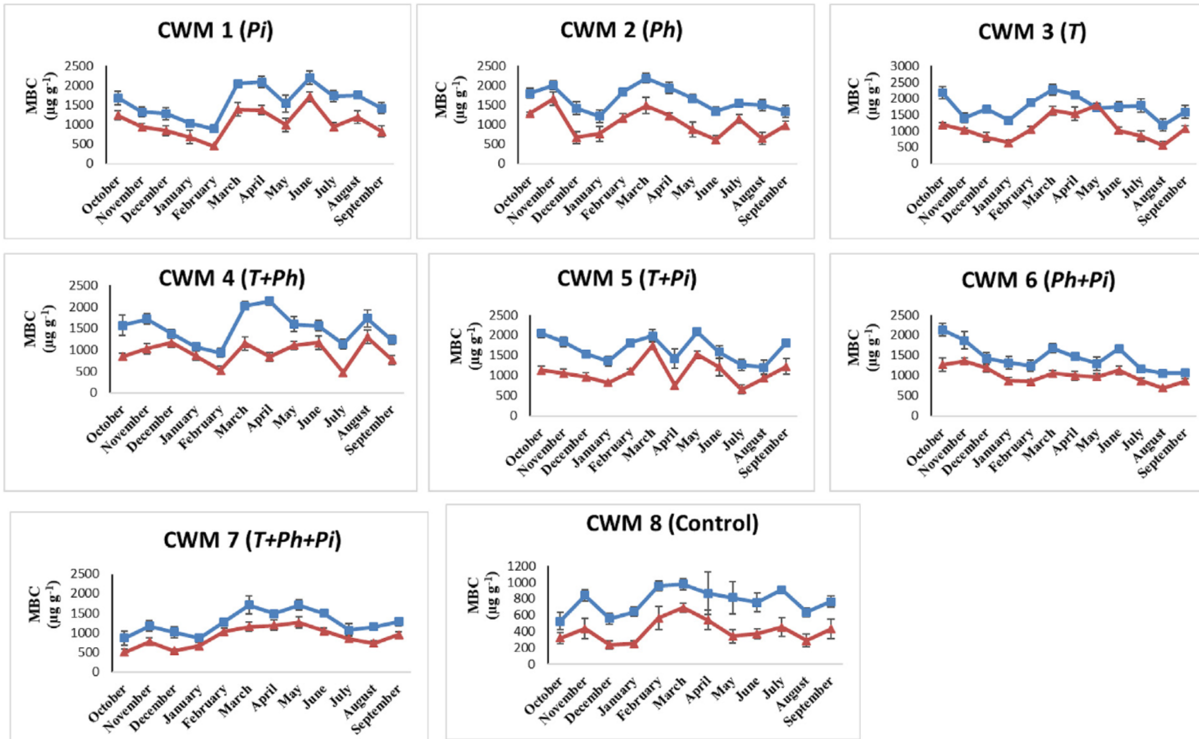

Fig. 7. Temporal variation in MBC at two consecutive soil layers in the macrophyte-specific CWM units (mean ± SD).

Pi and *T* + *Ph* + *Pi* at different sampling time. Urease activity at two consecutive soil depths and NO_3^--N removal efficiencies also have negative correlation except for CWM units with Ph and *T* + *Pi*. There was also negative correlation among activity of urease and NO_2^--N removal efficiencies at 3 and 7 d but positive at 14 d in most of the CWM units. Similarly, DHA and BOD removal efficiencies also exhibited negative correlation except CWM units with *T* + *Pi* and *T* + *Ph* + *Pi* at 3 d. A moderate positive and negative correlation between FDA hydrolysis and BOD removal efficiencies was observed with most of the CWM units showing positive correlation at 3 d and 14 d. However, CWM unit with *Ph* + *Pi* and *T* + *Ph* + *Pi* have significant negative correlation. Removal efficiencies of BOD were significantly correlated with MBC in CWM units with *Pi* and *T* + *Ph* + *Pi* and negatively correlated with each other in rest of the CWM units. According to macrophyte species, activity of phosphatase was strongly positively correlated with TP removal efficiencies in units with *Pistia stratiotes, Phragmites karka, Typha latifolia* as a single planting and *Phragmites karka* + *Typha latifolia* in mixed planting.

4. Discussion

Biomolecules have significant importance in transformation of nutrients in soil through their catalytic activity. Majority of the organics transformed in soil are catalyzed by enzymes (Jackson et al., 2013) and the transformation occurs by biochemical processes in soil substrate which are eventually reliant on, or linked to the enzymatic activity. The evaluation of soil enzyme activity has been done previously to describe microbial biomass (Klose and Tabatabai, 2000; Wang et al., 2007), microbial activity (Wang et al., 2007), bio-geochemical cycling of nutrients, as a potential source for bioremediation (Romaní et al., 2012) and to recognize the importance of rhizosphere effect (Chen et al., 2002). Amongst the study of various soil enzymes for several purposes, DHA, phosphatase and urease are well known enzymes because of their significant importance in transformation of organics and cycling of nutrients. Several previous findings have indicated that the activity of enzymes is inversely proportional to soil depth, i.e. decrease in activity of enzymes with increasing depth (Aon and Colaneri, 2001; Niemi et al., 2005; Kong et al., 2009). Similarly, in this study the activity of all enzymes was always maximum at the uppermost layer of soil substrate as compared to bottom layer in all CWM units. There was substantial variation among the activity of enzymes between both layers due to decrease in microbial populations, organic carbon, moisture and other inorganic nutrients in deeper layer. The difference was also due to different macrophyte-specific CWM units having free-floating and emergent macrophyte. Thus, the top layer (0–10 cm) of soil substrate within the CWM units is vital for the decomposition of pollutants. Relationship among pollutant removal efficiency and activity of enzymes vary with respect to pollutants and specific enzymes. Removal of TP was strongly positive correlated with phosphatase activity in almost all CWM units. SRP removal and phosphatase activity strongly depended upon macrophytic species. There are both positive and negative correlations. The richness and action of phosphatase enzymes in the soil substrate is a sign of the available phosphorus, accountable for alteration of organic phosphorus to inorganic and labile phosphorus forms through hydrolysis. It is a crucial oxido-reductase enzyme in soil substrate that has a significant role in phosphorus-cycle. Urease is generally considered as hydrolase enzyme accountable for hydrolytic alteration of the urea into ammonia and carbon dioxide. The assay of urease enzyme activity is vital to understand process of nitrogen mineralization. As discussed previously by Antia et al. (1991) and Thoren (2007), the non-enzymatic hydrolysis of urea compounds occurs actually at low rate (approx. 0–2%). This observation demonstrates the significant part of urease enzyme in the hydrolysis of urea. Urease enzyme showed significant positive and negative correlation with removal of NO_3^--N

Table 2
Correlation coefficients among enzyme activities and pollutant removal efficiencies in macrophyte-specific CWM units (no. of wastewater samples = 12 for each retention time and soil depth; no. of enzymes = 5).

Enzyme activities/Removal efficiencies	*Pi*		*Ph*		*T*		*T + Ph*		*T + Pi*		*Ph + Pi*		*T + Ph + Pi*		Control	
	0–10 cm	10–15 cm	0–10 cm	10–15 cm	0–10 cm	10–15 cm	0–10 cm	10–15 cm	0–10 cm	10–15 cm	0–10 cm	10–15 cm	0–10 cm	10–15 cm	0–10 cm	10–15 cm
Phosphatase- TP removal (3 d)	0.583	0.776**	0.737**	0.632*	0.212	0.362	−0.314	−0.455	0.004	−0.006	−0.341	−0.461	−0.201	−0.214	−0.612*	−0.361
Phosphatase- TP removal (7 d)	0.334	0.575*	0.029	0.309	0.765**	0.757**	0.688*	0.472	0.447	0.295	0.241	0.359	0.267	0.214	0.283	0.437
Phosphatase- TP removal (14 d)	0.211	0.011	0.657*	0.531	−0.223	−0.092	−0.055	0.040	−0.068	−0.258	−0.109	−0.282	−0.535	−0.523	−0.317	−0.282
Phosphatase- SRP removal (3 d)	0.432	0.262	0.306	0.313	−0.002	0.092	−0.809**	−0.615*	−0.315	−0.577*	0.006	−0.154	−0.672*	−0.616*	−0.259	−0.067
Phosphatase- SRP removal (7 d)	0.287	0.102	−0.219	−0.069	−0.259	−0.106	0.025	−0.513	−0.199	−0.110	0.495	0.501	−0.323	−0.343	0.624*	0.535
Phosphatase- SRP removal (14 d)	0.320	−0.034	−0.210	−0.272	−0.040	0.057	−0.007	0.001	0.031	0.072	0.087	0.209	−0.467	−0.467	−0.670*	−0.619*
Urease $-NH_4^+$-N removal (3 d)	−0.196	−0.049	−0.221	0.093	0.163	0.001	0.143	−0.252	0.231	0.254	0.112	−0.92	−0.005	−0.111	−0.397	−0.845**
Urease $-NH_4^+$-N removal (7 d)	−0.329	−0.384	0.202	0.023	0.182	0.484	0.396	0.327	0.009	0.336	0.504	0.414	0.736**	0.193	0.521	0.073
Urease $-NH_4^+$-N removal (14 d)	0.216	0.240	0.352	0.247	−0.011	0.123	0.287	0.471	0.363	−0.137	0.595*	0.744**	0.387	0.095	−0.400	−0.454
Urease $-NO_3^-$-N removal (3 d)	−0.470	−0.436	0.220	0.116	0.076	0.095	−0.125	−0.076	0.128	0.011	0.183	−0.002	−0.100	0.046	−0.070	−0.419
Urease $-NO_3^-$-N removal (7 d)	−0.360	−0.337	−0.048	−0.171	−0.035	−0.186	0.045	−0.146	0.083	0.227	0.313	0.094	−0.128	0.233	0.269	0.311
Urease $-NO_3^-$-N removal (14 d)	−0.349	−0.366	0.061	0.081	−0.207	0.010	−0.086	−0.148	0.021	0.181	−0.002	−0.247	−0.398	−0.010	−0.160	−0.195
Urease $-NO_2^-$-N removal (3 d)	−0.745**	−0.745**	−0.142	−0.336	−0.574	−0.108	0.148	0.071	−0.283	0.288	0.021	0.334	−0.272	−0.261	0.153	0.126
Urease $-NO_2^-$-N removal (7 d)	−0.215	−0.243	−0.335	−0.157	0.209	−0.022	0.275	−0.036	0.074	−0.023	0.013	−0.162	0.310	0.366	−0.226	−0.437
Urease $-NO_2^-$-N removal (14 d)	−0.436	−0.468	0.137	0.084	0.127	0.329	0.327	0.320	0.546	0.285	0.532	0.383	0.010	0.624*	−0.188	0.034
DHA- BOD removal (3 d)	−0.586	−0.402	−0.543	−0.433	−0.420	−0.263	−0.327	−0.355	−0.429	−0.520	−0.523	−0.240	−0.374	−0.135	0.050	−0.125
DHA- BOD removal (7 d)	0.006	−0.041	−0.058	−0.214	−0.227	−0.179	−0.216	−0.240	0.402	0.384	0.034	−0.080	0.035	0.280	−0.325	−0.101
DHA- BOD removal (14 d)	−0.125	−0.067	−0.460	−0.471	−0.196	−0.078	−0.289	−0.368	−0.173	−0.162	−0.029	0.126	−0.359	−0.316	−0.407	−0.174
FDA- BOD removal (3 d)	−0.378	−0.125	−0.501	−0.116	−0.041	0.011	−0.421	−0.516	−0.515	−0.131	−0.159	−0.648*	−0.257	−0.620*	−0.627*	−0.076
FDA- BOD removal (7 d)	0.205	0.020	0.014	−0.221	−0.165	−0.389	−0.072	0.044	0.198	0.396	0.295	0.330	0.088	−0.579*	−0.319	−0.617*
FDA- BOD removal (14 d)	0.044	0.138	−0.163	−0.569	0.392	0.339	0.044	−0.047	−0.237	−0.175	0.071	−0.039	−0.119	−0.327	−0.067	−0.539
MBC-BOD removal (3 d)	−0.550	−0.591*	0.038	−0.039	0.041	−0.196	0.241	0.507	−0.100	−0.171	0.167	0.415	−0.493	−0.578*	−0.321	−0.365
MBC- BOD removal (7 d)	0.468	0.419	−0.010	0.220	0.097	−0.592*	−0.128	−0.221	−0.307	−0.387	0.391	0.279	−0.631*	−0.700*	−0.184	−0.020
MBC- BOD removal (14 d)	−0.162	−0.352	0.216	0.477	0.224	0.024	0.108	0.081	−0.107	−0.398	−0.121	−0.17	−0.507	−0.589*	−0.240	−0.142

** Significant correlation at $p < 0.01$ level.
* Significant correlation at $p < 0.05$ level.

and NO_2^--N which further depended upon macrophyte species and retention time. It is assumed that the most of nitrogen in domestic wastewater is present in the form of NH_4^+ that limits the nitrification process due to lower dissolved oxygen. Therefore, a moderate correlation was observed among urease activity and NO_3^--N and NO_2^--N removal efficiency. Dehydrogenase enzyme in the soil substrates exhibited a key role in the early oxidation of soil organics via transporting H_2 or electron to acceptors from substrates (Phale et al., 2019). It is considered as a potential indicator of microbiological activity within the soil substrate. Correlation between BOD and DHA exhibited both positive and negative correlation depending upon macrophytes species, depth of soil substrate and time of sampling. The FDA hydrolysis in a soil sample evaluate the activity of microorganism's inhabitants and offers an estimate of complete microbial action. Overall activity of microbes in natural habitats offers an estimate turnover of organic matter as microbial decomposers are responsible for around 90% of the energy transfer in the soil ecosystem (Green et al., 2006). There was a moderate positive and negative correlation between FDA hydrolysis and BOD removal efficiencies with most of the CWM units showing positive correlation. However, some have significant negative correlation depending upon macrophytic species. Carbon sequestration in soil ecosystem has been measured as a possible potential source to mitigate climate change globally (IPCC, 2003; Smith et al., 2005) as well as for smooth functioning of ecosystem. For that, the microbial biomass is the prime constituent and it is also the main agent of the soil environment accountable for driving nutrient cycle and energy transfer (Megha and Simpy, 2014). They suggested that the approximately half of the microbial biomass is present in the top 10 cm layer of the soil samples and majority of transformation reactions occurs in this region that is also evaluated in the present study. Removal efficiencies of BOD were significantly correlated with MBC in CWM units with *Pi* and $T + Ph + Pi$ and negatively correlated with each other in rest of the CWM units. It is suggested by Kang et al. (1998) that activity of enzymes within soil sample is responsible for enhancement of water quality in wetland ecosystems. These extracellular soil enzymes showed dissimilar activity with respect to time, having highest and lowest peaks in different months of the year.

5. Conclusion

Nutrient cycling and conversion of organics in natural ecosystem utilizes several exogenous enzymes that are released by microbial population. Therefore, evaluation of the activity of these enzymes that have potential to decompose organics and mineralize nitrogen and phosphorus within soil substrate is very important. This study uses various macrophytic combinations to treat raw domestic wastewater, along with assessing activity of different enzymes at different soil depths in CWMs. The correlation of enzyme activities with efficiency of pollutants removal in mixed and single planting units of selected plant species has not been reported earlier. Several CWM units designed using emergent and free-floating macrophytes as a single as well as in combination provide effective mechanism for the treatment of domestic wastewater. Several mixed planting units showed higher enzyme activity as compared to units with single plantings. In the CWM units, vertical variation in activity of enzymes is observed, which indicated that the top layer of soil expressed significantly higher activity of these extracellular enzymes and were significantly different from deeper layers. Unit planted with *Pistia stratiotes* and *Phragmites karka* exhibited higher enzyme activity in the top as well in deeper soil layer along with higher removal efficiency for most of the wastewater pollutants. Most of the mixed CWM units showed significant positive correlation among enzyme activity and pollutant removal efficiency. However, temporal variation exhibited significant variations over time with higher activity in May, June, and October for most of the enzymes. For correlation study, the results concluded that the activity of phosphatase was strongly correlated with total phosphorus removal and SRP removal was moderately correlated with phosphatase activity. Urease activity and NH_4^+-N removal was also positively correlated with each other at most of the time with significant positive correlation in CWM units with $Ph + Pi$ and $T + Ph + Pi$. Activity of urease was also correlated positively and negatively with NO_3^--N and NO_2^--N removal in all CWM units. BOD removal was positively correlated with DHA, FDA hydrolysis and MBC on 7 d and 14 d respectively. From these findings, it is concluded that the activity of these extracellular enzymes within CWM units have crucial roles in removal performance of various contaminants of domestic wastewater.

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to authorship, research and publication of this paper.

CRediT authorship contribution statement

Saroj Kumar: Data curation, Methodology, Writing - original draft, Project administration, Investigation, Formal analysis. **Sampurna Nand:** Formal analysis, Validation, Visualization. **Divya Dubey:** Writing - review & editing. **Bhanu Pratap:** Formal analysis, Visualization. **Venkatesh Dutta:** Conceptualization, Supervision.

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Appendix A. Supplementary data

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REVIEW ARTICLE

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Constructed wetland microcosms as sustainable technology for domestic wastewater treatment: an overview

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Abstract

Constructed wetland microcosms (CWMs) are artificially designed ecosystem which utilizes both complex and ordinary interactions between supporting media, macrophytes, and microorganisms to treat almost all types of wastewater. CWMs are considered as green and sustainable techniques which require lower energy input, less operational and maintenance cost and provide critical ecological benefits such as wildlife habitat, aquaculture, groundwater recharge, flood control, recreational uses, and add aesthetic value. They are good alternatives to conventional treatment systems particularly for smaller communities as well as distant and decentralized locations. The pH, dissolved oxygen (DO), and temperature are the key controlling factors while several other parameters such as hydraulic loading rates (HLR), hydraulic retention time (HRT), diversity of macrophytes, supporting media, and water depth are critical to achieving better performance. From the literature survey, it is evaluated that the removal performance of CWMs can be improved significantly through recirculation of effluent and artificial aeration (intermittent). This review paper presents an assessment of CWMs as a sustainable option for treatment of wastewater nutrients, organics, and heavy metals from domestic wastewater. Initially, a concise note on the CWMs and their components are presented, followed by a description of treatment mechanisms, major constituents involved in the treatment process, and overall efficiency. Finally, the effects of ecological factors and challenges for their long-term operations are highlighted.

Keywords Constructed wetland microcosms · Domestic wastewater · Nutrients · Heavy metals · Macrophytes · Sustainability

Introduction

Lack of appropriate wastewater management practices are contributing to both scarcity and decline of fresh water quality worldwide (Almuktar et al. 2018). The situation is posing serious threat to ecosystems especially in developing countries (Wu et al. 2017). Discharge of majority of raw wastewater directly into rivers has become a common practice due to lack of suitable and effective technologies, operational failures of larger treatment plants, and higher cost involved in setting new treatment units (Kumwimba et al. 2017). The constructed wetlands (CWs) are engineered systems that have evolved as an inventive approach to tackle wastewater from domestic sources mainly because of their reliable efficiency, ecological benefits, easy operation, and less maintenance cost (He et al. 2018: Kumar and Dutta 2019). They use natural functions of macrophytes, soil, and microorganisms to treat different water streams (Ilyas and Masih 2017). The use of this technique has grown-up over recent decades with various successful examples (Zhang et al. 2014). CWs are being used to treat almost all types of wastewater such as domestic sewage, stormwater runoff, agricultural runoff, industrial drainage, and polluted rivers water (Li et al. 2017). There are many co-benefits of CWs together with wastewater treatment and recycling as they also provide important ecological services such as valuable wildlife habitat, aquaculture, groundwater recharge, carbon sequestration, fisheries, flood control, silt capture, recreational uses, and add aesthetic values to the surroundings.

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Classification of constructed wetlands CWs are characterized generally into three categories, namely, subsurface flow constructed wetlands (SSFCWs), surface flow constructed wetlands (SFCWs), and hybrid system. Further, on the basis of the flow path, SSFCWs are differentiated into vertical flow constructed wetlands (VFCWs) and horizontal flow constructed wetlands (HFCWs) (Wang et al. 2018). According to the macrophytic growth, they are categorized into emergent, free-floating, submerged, and floating-leaved macrophytes (Vymazal 2010).

Constructed wetland microcosms (CWMs) A working model of a CWM (Fig. 1) possesses various types of supporting media and aquatic macrophytes depending upon target pollutants. In general, wastewater reaches the treatment chamber, runs all the way through the supporting media, and is released out of the chamber from an outlet system. A CWM unit has following five major components: basin (or chamber), substrate/media materials, vegetation (mostly macrophytes), and inlet and outlet system (Sudarsan et al. 2015).

A number of researchers across the world have published their review articles on the use of CWs for wastewater treatment (Liu et al. 2015; Haynes 2015; Almuktar et al. 2018). However, there are somewhat few studies detailing the treatment dynamics, rather the information is meant to provide onsite domestic wastewater treatment that are site specific. Recent investigation on CWs has principally provided information on wastewater decontamination (Avila et al. 2014), suitable working models and appropriate choice of macrophytes (Wang and Sample 2013), retention time (HRT), hydraulic loads (HLR) (Dzakpasu et al. 2015), and variety of supporting media (Ge et al. 2015) (Fig. 2).

Treatment mechanisms involved in CWMs

Treatment mechanisms involved in CWMs are biogeochemical transformations and solid/liquid separations. Transformation possesses reduction, oxidation, acid/base reactions, biochemical reactions, flocculation, and precipitation. Separation includes adsorption, absorption, gravity separation, stripping, leaching, filtration, and ion exchange (Choudhary et al. 2011).

Major constituents involved in treatment mechanisms

Wetland vegetation (macrophytes)

In CWMs, macrophytes are primary vegetation. They are essentially grouped in four categories, namely, emergent, submerged, floating-leaved, and free-floating macrophytes (Kumar and Dutta 2019). Growth characteristics and nutrient uptake capacity of some frequently used macrophytes are presented in Table 1. The macrophytes relocate oxygen and provide dissolved organic matter and supporting media for microbial attachment (Meng et al. 2014). They are also contributing to enhance porosity and permeability of the substrate, act as a catalyst, and promote a number of biological and chemical reactions (Yahiaoui et al. 2018). More than 150 species of macrophytes have been reported that are used in CWMs worldwide; however, only a few of them are commonly used. It is observed that emergent aquatic macrophytes are preferred choice because they have high contaminant removal efficiency (Vymazal 2013). The choice of macrophytes must be indigenous which can grow naturally in wetlands. They should be also capable to withstand with short dry periods as well as shocks generated by wastewater loads. Macrophytes which have well developed root and rhizome systems inside the supportive material are most preferable.

Supporting media

Currently, available and frequently used supportive media are the industrial by-products, natural and artificial or synthetic materials (Yan and Xu 2014). Some frequently used supporting media in CWMs are presented in Table 2. They must be chosen according to their capacity to absorb/adhere wastewater contaminants and their permeability. It is generally observed that reduced hydraulic conductivity greatly influenced adsorption ability (Wang et al. 2010). Ultimately, the long-lasting applications of the treatment system are highly affected by the chosen media materials (Wang et al. 2010).

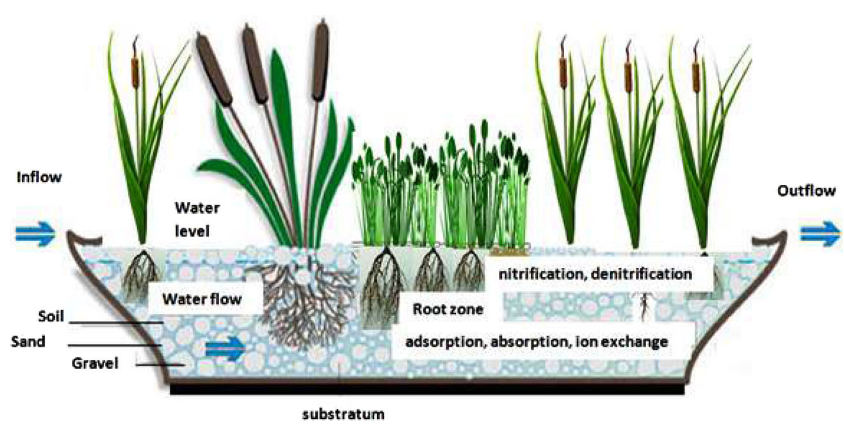

Fig. 1 CWM unit planted with emergent macrophytes

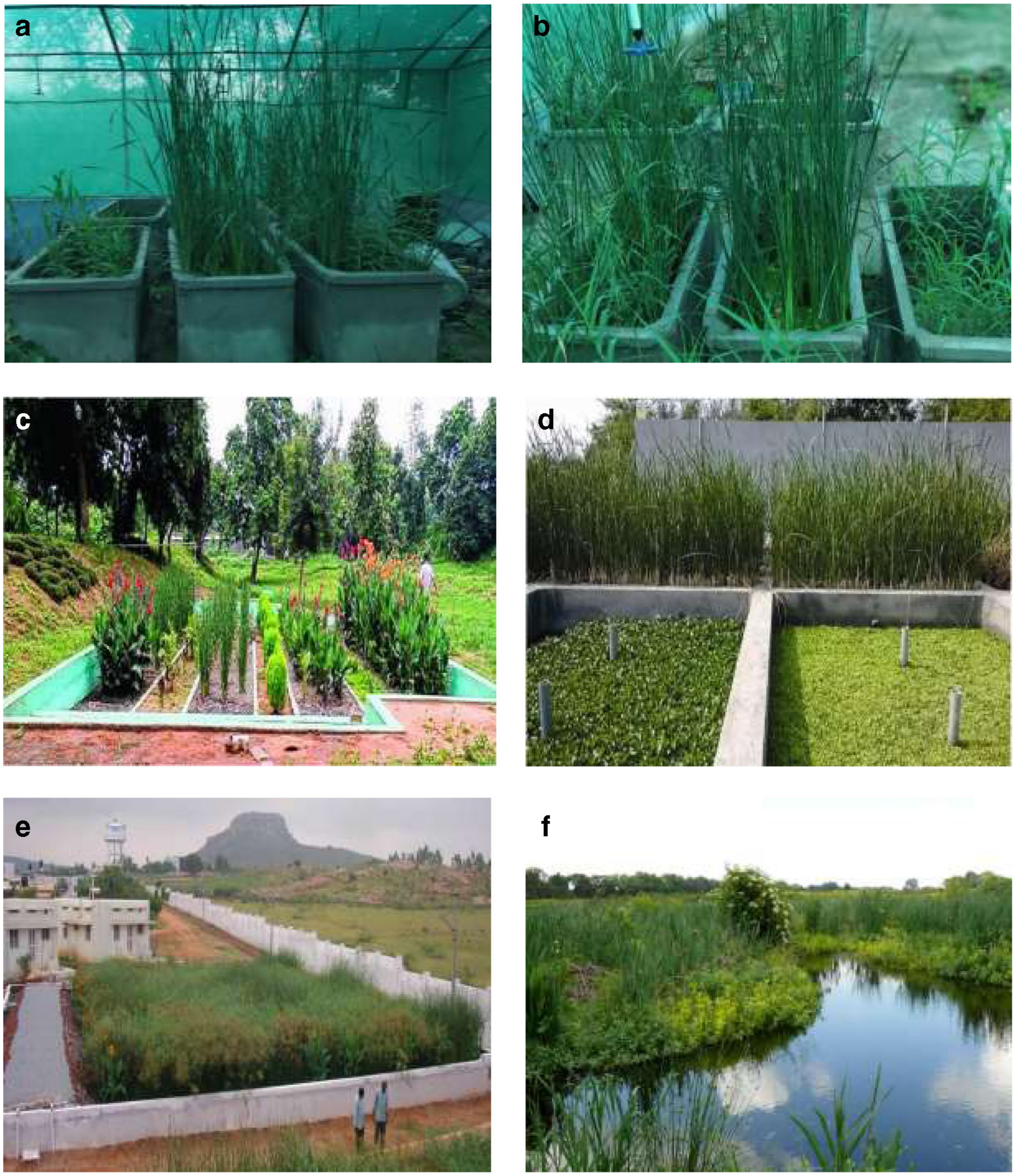

Fig. 2 **a**, **b** CWM units designed under net house of Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India. **c** CSIR- Institute of Minerals and Materials Technology, Bhubaneswar, Odisha. **d** International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India. **e** Constructed wetland for wastewater treatment for a colony in Andhra Pradesh, India. **f** CWs working successfully in Georgia treating runoff from a plant nursery

Microorganisms

The principal microorganisms concerned with wetlands system are bacteria, yeasts, protozoa, fungi, and algae. Collectively, all these microorganisms participate in the degradation of nearly all of the wastewater contaminants into insoluble or harmless substances. The well-established microbial communities are attached to the supporting media, plant roots, and/or in leaves in the form of biofilms (Faulwetter et al. 2009). The complex microbial communities in the form of biofilms formed by interactions with wastewater are primarily responsible for the breakdown of the wastewater pollutants and increase the overall treatment performance of the CWMs (Sleytr et al. 2009). Several previous studies have identified and characterized microbial communities in full-scale constructed wetlands and laboratory scale units under

Table 1 Growth characteristics of some frequently used aquatic macrophytes in CWMs treating municipal wastewater

Type	Macrophytes	Optimal temperature (°C)	Optimal pH	Root penetration (cm)	Maximum water depth (in.)	Growth	Drought resistance	Nutrient uptake capabilities ($kg\ ha^{-1}\ yr^{-1}$)	
								Nitrogen	Phosphorous
Emergent	*Phragmites* sp. (common reed)	12–23	3.7–8	60	3	Very rapid	High	2500	120
Emergent	*Typha* sp. (cattail)	10–30	4–10	75	12–18	Rapid	Possible	1000	180
Emergent	*Scirpus* sp. (bulrush)	16–27	4–9	75	12	Moderate to rapid	Moderate	–	–
Emergent	*Juncus* sp. (rush)	16–26	5–7.5	25	3	Rapid	Moderate	–	–
Free-floating	*Lemna* sp. (duckweed)	6–33	6.5–7.5	2	19	Very rapid	No	–	–
Free-floating	*Pistia stratiotes* (water lettuce)	15–35	6–6.8	80	–	Very rapid	No	900	40
Free-floating	*Eichhornia crassipes* (water hyacinth)	12–35	6.5–7.5	100	–	Very rapid	No	2400	350

*Compiled from various sources

specific environments (Calheiros et al. 2009; Krasnits et al. 2009; Sleytr et al. 2009; Dong and Reddy 2010; Zhang et al. 2010). However, in case of domestic wastewater, there is lack of information about how the microbial communities and diversity change during long-term operations (Adrados et al. 2014). Comprehensive information about the structure of these communities must be attained by suitable design improvisation in order to understand the biological developments that are taking place inside them (Dong and Reddy 2010). It is observed that the rhizosphere region of the CWMs is capable of providing unique add-on sites for microbial connection and release root exudates and oxygen which helps in estimating the role of the microbial cosmos (Zhang et al. 2016; Lv et al. 2017). Different design and operational parameters undertaken to treat various wastewater in several countries are presented in Table 3.

Removal of organics

Biodegradation of organics takes place by both aerobic as well as anaerobic microorganisms depending upon the availability of oxygen. For aerobic degradation, oxygen can be added from convection, atmospheric dispersal and through root organization of macrophytes (Cooper et al. 1996), while pores of supporting media are sites responsible for anaerobic biodegradation. Settleable organics are removed rapidly under gravitational forces by filtration and sedimentation whereas soluble organics are removed by attached or suspended microbial growth. Degradation of organics by aerobic processes mainly proceeds by aerobic chemoheterotrophs because they have a faster metabolic rate as compared to chemoautotrophs. These chemoheterotrophic bacteria oxidize organic compounds using oxygen and release carbon dioxide (CO_2), ammonia (NH_3), and other stable compounds (Garcia et al. 2010). Sufficient supply of oxygen greatly enhances degradation of organic matter by increasing biochemical oxidation (Vymazal and Kropfelova 2009). Anaerobic degradation of organic matter by anaerobic heterotrophic bacteria involves two processes namely methanogenesis and fermentation. In methanogenesis, methanogens (methane-producing bacteria) convert organic compounds into methane (CH_4) and CO_2 and produce new bacterial cells whereas fermentation utilizes acid-forming bacteria to convert organic matter into organic acids and alcohols. These two processes continue in anaerobic zone of wetland system (Kadlec and Knight 1996).

Removal of nitrogen

The contribution of macrophytes in terms of nitrogen removal varies among several species such as *Typha latifolia* contributing 1.73 to 8.81%, *Canna indica* 0.98 to 17.95%, and for

Table 2 Frequently used supportive media in CWMs (Revised from Wu et al. 2015)

S. no.	Supporting media type	Type of wastewater*	Reference
1	Industrial by-products		
	Fly ash	Municipal	Xu et al. 2006
	Coal cinder	Domestic	Ren et al. 2007
	Slag	Domestic	Zuo et al. 2018
	Alum sludge	Synthetic	Babatunde et al. 2010
	Oil palm shell	Synthetic	Chong et al. 2013
	Hollow brick crumbs	Domestic	Ren et al. 2007
2	Natural material		
	Sand	Textile	Saeed and Sun 2013
	Gravel	Tannery	Lima et al. 2018
	Clay	Tannery	Calheiros et al. 2008
	Limestone	Synthetic	Tao and Wang 2009
	Zeolite	Municipal	Bruch et al. 2011
	Maerl	Synthetic	Saeed and Sun 2012
	Shale	Synthetic	Saeed and Sun 2012
	Peat	Domestic	Saeed and Sun 2012
	Organic wood mulch	Synthetic	Saeed and Sun 2012
3	Artificial material		
	Compost	Refinery	Saeed and Sun 2012
	Activated carbon	Domestic	Ren et al. 2007
	Lightweight aggregates	Synthetic	Lima et al. 2018
	Basic oxygen furnace slag (BOFS)	Synthetic	Barca et al. 2014
	Rice straw	Hypereutrophic water‡	Cao and Zhang 2014
	Light ceramsite	Hypereutrophic water	Cao and Zhang 2014
	Electro-oxidation	Hypereutrophic water	Cao and Zhang 2014

*Domestic wastewater has been used here to include wastewater originating from household activities from a community whereas municipal wastewater is generated in towns and urban areas from any combination of domestic, commercial, or agricultural activities including wastewater from public facilities, surface runoff, stormwater, and any sewer inflow or sewer infiltration. Industrial wastewater is a by-product of industrial or commercial activities. Synthetic or artificial wastewater differs from domestic wastewater or municipal wastewater as it is synthetically made according to the treatment technologies to be tested

‡Hypereutrophic water is sourced from a lake or other water body characterized by excessive nutrient concentrations (nitrogen and phosphorous) and high productivity

Phragmites australis, it ranges from 7.15 to 17.04% (Jesus et al. 2018). In CWMs, the different macrophytes offer oxygen and surface which is necessary for the development of microbes in the root zone, consequently enhancing nitrification. In addition, there is supply of carbon from root system (5–25%, fixed photosynthetically) and optimization of denitrification process (Wang et al. 2012). Wastewater stream has typically inorganic and/or organic form of nitrogen (Stefanakis et al. 2014). Major nitrogen elimination pathways which are engaged with CWMs are classified into two broad categories—novel (new) and classical (traditional) nitrogen removal pathways (Saeed and Sun 2012). Traditional nitrogen removal pathways in CWMs include ammonification, ammonia volatilization, nitrification, denitrification, and adsorption. In the CWM system, ammonification is more in the upper aerobic facultative zone as compared to the bottom obligate anaerobic zone. Both ammonification and ammonia volatilization are pH-dependent process. The suggested pH value to get good results from ammonification ranges from 6.5–8.5 (Saeed and Sun 2012), while a notable rise in pH (> 9.3) converts ammonium ions into ammonia gas (Bialowiec et al. 2011). Adsorption takes place mostly in the form of ammonia into the supporting media (Tsihrintzis 2017) which is used to encourage cation exchange capacity. Supporting media with greater cation exchange capacity has been employed due to their enhanced nitrogen removal efficiency (Saeed and Sun 2012). Biochar is a potential material which supports the denitrification process and removal of NO_3^- by providing organic carbon source. A short description of novel nitrogen removal pathways is provided below:

Table 3 Wetland design and operational parameters considered for different wastewater in several countries

Type of wastewater (WW)	Total surface area m^2	Plant species	Plant density plants m^2	HLR $m^3/m^2/day$	HRT days	Flow rate m^3/d	Study area/country	Reference
Municipal	185.5	*C. papyrus*	NA	0.18, 0.10, and 0.07	1.8, 3.2, and 4.7	8	Giza, Egypt	Abou-Elela et al. 2017
Municipal	1.5	*P. australis*	NA	0.37	1.75	0.75	Barcelona, Spain	Avila et al. 2017
Domestic	1.09	*H. psittacorum*	NA	0.15	NA	0.150	Pereira, Colombia	Bohórquez et al. 2016
Domestic	200	*P. australis* *T. latifolia*	4	0.46 0.1	0.7 1.2	65 76	Bedfordshire, UK	Butterworth et al. 2016
Secondary*	0.66	*C. articulatus*	33	0.46	3	0.028	Atlántico, Colombia	Caselles-Osorio et al. 2017
Domestic	0.6	*C. ligularis* *E. colona*	38	0.06	2.3	0.042	Barranquilla, Colombia	Casierra-Martínez et al. 2017
Domestic	130	*P. australis*	4	0.5 and 0.75	NA	NA	Marrakech, Morocco	Elfanssi et al. 2017
Domestic	45.36	*T. parviflora*, *J. acutus*, *S. perrenis*, *L. monopetalum*	1	0.053	3.48	2.4	Heraklion, Greece	Fountoulakis et al. 2017a
Domestic	1.08	*A. halimus*, *J. acutus* and *S. perennis*	9	0.095	NA	0.6	Heraklion, Greece	Fountoulakis et al. 2017b
Synthetic	0.137	*R. japonicas*, *O. hookeri*, *P. arundinacea* and *R. carnea*	12	NA	10	NA	Beijing, China	Geng et al. 2017
Domestic	180	*C. generalis*	4–5	1728	0.25	NA	Udupi District, India	Ojoawo et al. 2015
Urban	51.87	*T. latifolia, P. australis*, and *C. esculenta*	NA	NA	2–3	NA	Haridwar, India	Rai et al. 2015
Municipal	0.0004	*L. perenne*	0.1	0.0375	6	0.15	Xian, China	Ren et al. 2016
Domestic	404	*T. latifolia* L and *S. tabernaemontani*	NA	0.022	NA	NA	Ontario, Canada	Rozema et al. 2016
Synthetic	0.2	*E. crassipes*	NA	NA	2	0.012	Parana, Brazil	Lima et al. 2018
Domestic	30	*P. stratiotes*, *T. latifolia*, *C. indica*, and *A. conyzoides*	NA	0.10	5	3	Telangana, India	Tilak et al. 2017
Domestic	0.13	*P. australis*	8	NA	6	0.21	Shaanxi, China	Wu et al. 2016
Secondary	8,660,000	*P. australis*, *T. orientalis*, *Z. latifolia*, *N. nucifera*, *N. tetragona*, *P. crispus*, *L. minor* and *E. crassipes*	NA	0.035	7	380,000	Shaanxi, China	Wu et al. 2017
Synthetic	0.72	*T. angustifolia*	14–15	0.056	4	0.02	Singapore	Zhang et al. 2012
Synthetic	0.19	*R. japonica*, *O. javanica. P. arundinacea* L. and *J. effusus* L	12	NA	NA	NA	Hangzhou, China	Zhao et al. 2016

*Secondary wastewater is primary-treated wastewater

Novel nitrogen removal pathways

Recently, some new and more efficient nitrogen exclusion routes are pointed out which comprises of partial nitrification-denitrification, anaerobic ammonium oxidation (Anammox), and completely autotrophic nitrite removal (Canon). The main operating factors of partial nitrification processes (i.e., Anammox and Canon) include temperature, pH, free ammonia, free nitrous acid, HRT, dissolved oxygen, salt, organic compounds, and hydroxylamine (Wang and Yang 2004; Lee et al. 2009). They are described briefly in the following section.

Partial nitrification-denitrification This process involves translation of NH_4–N to NO_2–N which is called nitrification (Eq. 1) after that the denitrification of NO_2–N to N_2 gas (Eq. 2) takes place.

$$NH_4{}^{+} + 1.5O_2 \rightarrow NO_2{}^{-} + H_2O + 2H^{+} \tag{1}$$

$$NO_2{}^{-} + 1/2CH_3OH + H^{+} \rightarrow 1/2N_2 + 1/2CO_2 + 1.5H_2O \tag{2}$$

Jianlong and Ning (2004) reported that this process needs approximately 40% and 25% lower organics and oxygen respectively, as compared to other available nitrogen removal methods.

Anammox Oxidation of ammonium anaerobically (anammox) is a recently revealed nitrogen removal pathway in which ammonium changes into nitrogen gas with the assistance of *Planctomycetes* bacterial group under anaerobic environment. The anammox process is more advantageous than another treatment system as it requires external carbon in negligible amount. Further, oxygen and energy requirements are also very low and nitrogen is removed at greater speed (Saeed and Sun 2012).

Canon Removal of nitrite over nitrate in the complete autotrophic way involves the anammox process and partial nitrification simultaneously; together, these processes remove all available total nitrogen (TN) in a particular region. There is a mutual co-existence between anammox bacteria and ammonium oxidizing bacteria. Sun and Austin (2007) reported that the canon process in a vertical flow constructed wetlands (VFCWs) removed a significant amount of nitrogen (approximately 52%).

Removal of Total phosphate (TP)

A mixture of inert and natural phosphate is available in the wastewater stream, out of which, the most common is orthophosphates ($PO4^{3-}$). The performance of CWMs is reduced due to low phosphorus removal efficiency. The treatment efficiency of CWMs towards phosphate depends on the prevailing ecological situations, type and the number of macrophytes, available form of phosphate, and the loading rates (USEPA 2000). The contribution of macrophytes in removal of phosphate ranges from 4.8 to 74.87% (Jesus et al. 2018). Various macrophytes possess different plant uptake capacity such as *Typha latifolia* contributing 0.06 up to 74.87%, for *Canna indica*, 0.43 to 4.17%, and for *Phragmites australis*, it ranges from 0.56 to 36.7% (Jesus et al. 2018). It is pointed out that the higher water depth with reduced flow velocity advances the removal rate (Guo et al. 2017). Phosphate removal is regulated by immobilization by microorganisms, the adherence capability of a range of filter media used in different seasons, temperature, and growth periods. Dissolved state of phosphorus is taken up by macrophytes or adhered to the substrates when the cations such as Fe, Al, Mg, and Ca are present in excess. The process starts by ligand exchange reactions. Phosphate allocates H_2O and OH^{-} ions on the face of iron oxides and aluminum. However, the rate of deletion typically decreases unless an appropriate adsorbent matter is incorporated in the system (Vymazal 2010). Removal of phosphorus through various supporting media is ranging between 40 and 60%. Currently, a number of specialized media materials are used in CWMs to attain enhanced removal performance such as slag (Okochi and McMartin 2011), basic oxygen furnace slag (BOFS), sandstone, zeolite, dolomite bauxite (Stefanakis et al. 2014), and electric arc furnace (EAF) (Barca et al. 2014). It is reported that biochar has huge potential to enhance phosphorus removal by providing maximum adherence sites. Inorganic, organic, dissolved, and insoluble phosphate is not as such taken up by macrophytes until they are transformed into a simple soluble form (Choudhary et al. 2011). It has been observed that magnesium (Mg)-containing materials such as magnesia and magnesite, in the supporting media improves TP removal performance (Lan et al. 2018). In terms of plant uptake, macrophytes have lower phosphorus uptake capacity compared to nitrogen because

a. Under aerobic setting, unsolvable phosphate is precipitated with Fe, Ca, and Al ions.
b. Organic peat, clay, and Fe and Al hydroxides and oxides have participated in phosphate adsorption.
c. Phosphorus is bound up in organic matter through assimilation by bacteria, algae, and macrophytes.

A number of man-made substrates such as zirconium oxide nanoparticle (ZON), magnetic iron oxide nanoparticle (MION), and iron oxide coated granular activated carbon

(Fe-GAC) have been identified with improved adsorption capability. Because of high-cost involvement, discharge of secondary contaminants, and complications in manufacturing processes, the use of these materials is limited in full-scale treatment systems (Park et al. 2017). As a result, the selection of right filter media with better adsorption ability is crucial for better performance.

Removal of heavy metals

Wastewater which is contaminated with trace metals has the great impact on biosphere; therefore, the remediation of these trace metals is essential. The presences of such metals greatly affect the flora and fauna of an aquatic system (Parnian et al. 2016). Remediation of wastewater polluted with heavy metals implies various technologies in which adsorption, reverse-osmosis, electrodialysis, and ion exchange are more common. Almost all of such technologies are expensive, energy-intensive, and generally metals-specific. However, macrophytes in the CWMs are known to have the huge potential towards trace metals buildup in their tissues (Mishra and Tripathi 2008). Removal of metals from domestic wastewater through CWMs involves mainly filtration, sedimentation, adsorption, cation exchange, precipitation, complexation, macrophyte uptake, and microbial oxidation/reduction processes. Several biotic, abiotic, and environmental factors like pH and temperature in the CWMs have direct consequences on bioaccumulation of trace metals (Xing et al. 2013). Removal of heavy metals in CWs using aquatic macrophytes by different studies has been shown in Table 4.

Sustainability of CWMs

A sustainable design of CWMs for domestic wastewater treatment includes the suitable design of CWMs at proper site with efficient macrophytes and supporting media. Design in a way that it acquires the natural features of the surroundings and to diminish its disturbance. The working model is set by the prevailing landscape, geology, and availability of land. Supply of additional oxygen is via artificial aeration, water

Table 4 Removal of heavy metals in CWs using aquatic macrophytes

CWs type	Wastewater type	Plants used	Target metals	Removal efficiencies (%)		Study area/country	References
				Winter	Summer		
HFCW	Urban	*T. latifolia*	Pb, Cu Zn, Co	78.5, 72.5 68.4, 65.1	86, 84.0 83.4, 76.8	Haridwar, India	Rai et al. 2015
			Cr, As	64.5, 63.2	81.6, 82.2		
			Mn, Ni	53.3, 51.4	62.2, 68.1		
NA	Municipal	*E. crassipes*	Hg,	Up to 95		Irbid, Jordan	Qasaimeh et al. 2015
HFCW	Municipal	*P. phalaris*	Cu, Pd Ni, Zn	84, 78 46, 86		Brehov, Czech Republic	Kropfelova et al. 2009
			Hg	39			
HFCW	Domestic	*P. phalaris*	Cu, Pd Ni, Zn	84, 88 12, 87		Leon, Spain	Pedescoll et al. 2015
HFCW	Domestic	*P. australis*	Cu, Pd Ni, Zn	88, 67 36, 86		Zemst, Belgium	Lesage et al. 2007
HFCW	Municipal	*P. phalaris*	Cu, Pd Ni, Zn	73.8, 84.2 49.1, 90.5		Morina, Czech Republic	Kropfelova et al. 2009
			Hg	29.4			
VFCW	Synthetic	*C. indica*	Cr Ni	98.3 96.2		Bhubaneswar, India	Yadav et al. 2010
FWS	Rainfall	*P. australis*, *T. latifolia*	Cu, Pd Zn, Cd	60, 31 86, 05		Dublin, Ireland	Gill et al. 2017
NA	Synthetic	*P. stratiotes*	Pd Cr	13.0–84.3 92.0–95.0		Parana, Brazil	Lima et al. 2013
NA	Synthetic	*S. grossus*	Pd	99		Selangor, Malaysia	Tangahu et al. 2013
NA	Municipal	*P. australis* and *T. latifolia*	Cu, Cd Cr, Ni	78, 60 68, 73.8		Varanasi, India	Kumari and Tripathi 2014
			Fe, Pb, Zn	80.1, 61, 61			

NA, not available

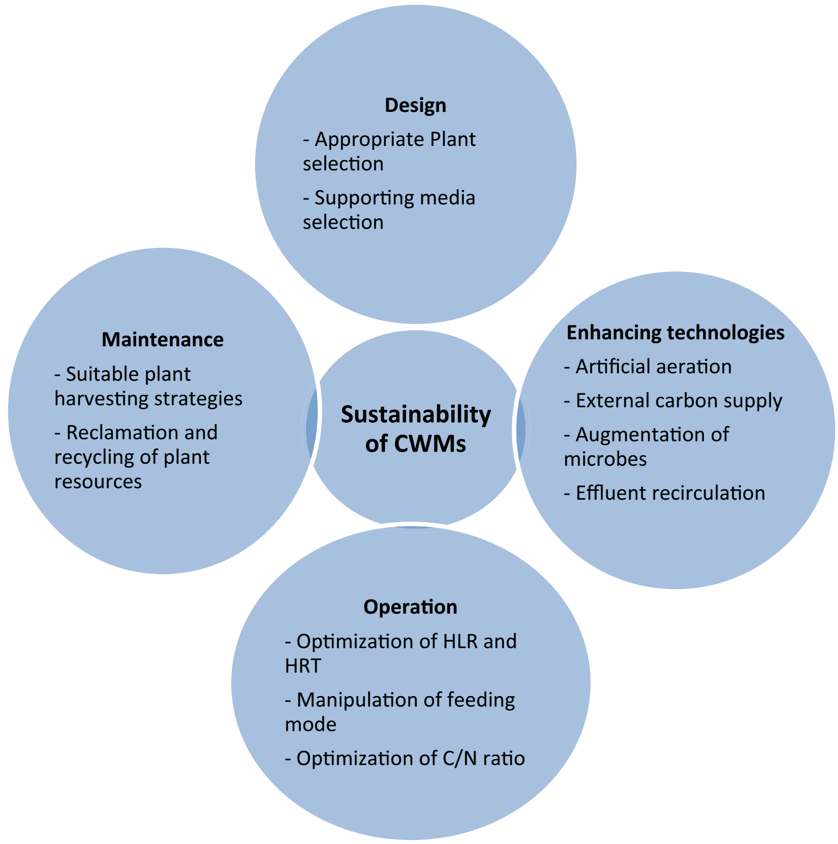

Fig. 3 Sustainability of CWMs—key criteria (modified from Wu et al. 2015)

depth, optimization of HLR and HRT, bioaugmentation of specific microorganisms, proper plant harvesting; reuse/recycling methods, and the addition of extra organic matters (Fig. 3) (Kadlec and Wallace 2009). Recently, the recirculation of effluent within the CWM system attains huge potential towards enhancement of removal performance through sufficient settling time. The removal performance of CWMs declines considerably when the environmental parameters such as water temperature, pH, and DO are not properly managed (Kadlec and Wallace 2008).

Future concerns and challenges

Firstly, optimization of hydraulics, selection of appropriate macrophytic species and supportive media, mode of operation, and pollutant loading rate are important factors to gain higher removal efficiencies. Suitable plant harvest techniques are vital because when they die and decay, leave nutrients and several other contaminants into the water body. In future research, there is a need to develop techniques to improve treatment efficiencies which could be achieved by microbial augmentation, artificial aeration, a range of supporting media, and supply of additional carbon, tidal action, step feeding, baffled flow, and mixed systems (Wu et al. 2015). CWMs are land intensive, requiring large land area and prone to seasonal weather conditions. Therefore, suitable design improvisation could be done to reduce the overall land requirements. This is also reported by various researchers that the CWMs are by nature prime mosquito habitat. This challenge could be tackled by conserving natural enemies (invertebrates) such as dragonflies, damselflies, beetles, predatory flatworms, true bugs, and crustaceans such as copepods, tadpole shrimp. Fishes, amphibians, spiders, bats, and microbial larvicide *Bacillus thuringensis* var. *israelensis (Bti)* are also used to control mosquitoes' larvae (Mazzacano and Black 2013).

Conclusion

CWMs can be designed as biofilters to imitate the features of natural wetlands for removing nutrients, and other contaminants from the wastewater streams. The focus of this review paper has been on evaluation of treatment performance of CWMs treating domestic wastewater. Both ecological factors such as temperature, pH, DO, and working parameters such as availability of carbon, HLR, HRT, pollutant loads, recirculation, C/N ratios, plant harvesting techniques, addition of extra organic matter, and bioaugmentation of specific microorganisms are vital to achieving sustainable contaminant removal efficiency. Supply of additional oxygen via artificial aeration (mainly intermittent) and effluent recirculation greatly enhances the removal efficiency for organics and nutrients. Novel nitrogen removal pathways have greatly enhanced the nitrogen removal. The removal efficiency increased at influent C/N ratio between 1 and 3 and decreased significantly at the increasing C/N ratios between 3 and 15. The contribution of macrophytes in terms of nitrogen removal varies from 0.98 to 93% and for phosphate ranges from 4.8 to 74.87% depending upon area of the root surface and root oxidizing capacity. Removal of phosphate mostly occurs by adsorption and its efficiency is usually low until a suitable supporting media is not incorporated. Biochar has great potential to support denitrification rate and NO_3–N removal by providing carbon source and also enhance phosphorus removal. Typically, the removal of phosphorus from a variety of supportive media ranged from 40 to 60%. Removal of heavy metals from wastewater implies various technologies such as ion exchange, electrodialysis, adsorption, and reverse-osmosis. Almost all these technologies are expensive metals-specific and energy-intensive. However, macrophytes are known to have huge potential towards trace metals buildup in their tissues.

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REVIEW

Removal of nutrients from domestic wastewater using constructed wetlands: assessment of suitable environmental and operational conditions

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Abstract
Constructed wetlands (CWs) offer an eco-friendly wastewater treatment technology primarily for decentralized locations. They support a dense growth of macrophytes which help in the reduction of water velocity, development of conducive micro-environments and provide adherence sites for microorganisms to develop biofilms. Several environmental and operational parameters are crucial for the efficient working of CWs wherein, suitable pH, temperature and dissolved oxygen (DO) are more significant. The performance of CWs has been enhanced significantly through effluent recirculation and artificial aeration. Removal of phosphorus proceeds via adsorption within media material, sedimentation, cation exchange, precipitation and uptake by macrophytes and the removal of nitrogen occurs mainly by microbial communities and macrophytic uptake. In colder climates, the bioaugmentation of microbial communities is required to increase the treatment efficiency. Various previous research findings showed several aspects of CWs that could potentially affect removal efficiency of the nutrients from the wastewater. However, maintaining suitable environmental and operational conditions for the effective operation of CWs remains a challenge. Therefore, the objectives of this review-based study are to provide the most favorable environmental conditions for the effective operation of CWs. Recent developments in operational and working parameters of CWs focusing on the selection of macrophytes and substrate material, carbon source, feeding mode, hydraulic loading rates and retention times are discussed. The study also provides effect of effluent recirculation, bioaugmentation of microbes, suitable C/N ratio and artificial aeration for the domestic wastewater treatment.

Keywords Constructed wetlands · Domestic wastewater · Macrophytes · Sustainability · Treatment efficiency

Introduction

Due to the lack of proper management and efficient treatment technology particularly in decentralized and remote locations, the majority of untreated wastewater is discharged directly into nearby water bodies (Kumar and Dutta 2019a,b). These practices cause severe contamination of both surface as well as groundwater. The situation becomes worse with rapid urbanization (Abou-Elela et al. 2017). Various conventional technologies such as membrane bioreactors, activated sludge process and membrane separation are applied successfully for the management of domestic wastewater in metropolitan cities. Due to high cost involvement, the use of these technologies is not practical on commercial scale especially in decentralized systems (Zhang et al. 2020). Constructed wetlands (CWs) are man-made ecosystems utilized for treatment of several types of wastewater (Kumar and Dutta 2019a; Kumar et al. 2020). These systems are environment friendly, cost-effective, easily operated and maintained and very effective in the removal of wastewater contaminants. Currently, the application of CWs have been stretched globally due to the enhancement in their configurations and operational features and they offer a more aesthetic

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technology as compared to conventional treatment methods (de Rozari et al. 2018). It is also known that a slight change in wetlands hydrology significantly affects its treatment performance. A CW has three key components: aquatic plants, substrate material and associated microbial population (Fig. 1). Together, all these play a significant role in the efficient working of a CW system (Kumar et al. 2020). Besides these, several environmental and operational parameters are also crucial for the smooth functioning of CWs. The environmental parameters such as pH, dissolved oxygen (DO) and temperature are vital. Operational parameters such as suitable design, appropriate site and macrophyte selection, effect of C/N ratio, feeding mode, artificial aeration, wastewater recirculation, suitable plant harvesting techniques, bioaugmentation of microbial populations, HLR (hydraulic loading rates) and HRT (hydraulic retention time) are also very crucial. Almost all wetland system promote aquatic life and sustain the growth of numerous aquatic plants. These plants reduce the water velocities and offer adherence sites for microorganisms by creating microhabitats inside the water column. After the death and decay of plant parts, litter provides additional carbon and nutrients required for microbial growth for further action and treatment.

Removal of wastewater nutrients in CWs occurs mainly through physical and biological processes. Physical processes involve sedimentation with the assistance of macrophytes and filtration with the help of supportive media (Sundaravadivel and Vigneswaran 2001). The main biological processes include nitrification, denitrification, fermentation, respiration and photosynthesis. Photosynthesis supports to maintain the supply of oxygen for macrophytes. Nitrification and denitrification are mechanisms of the nitrogen cycle resulting into the removal of nitrogen (N). Fermentation helps in the breakdown of organics. Respiration tends to maintain the DO content in the wetland systems. Adsorption is the key mechanism for the removal of phosphorus which occurs when ions of calcium, magnesium and iron react with phosphorus present in the substrate material. Adsorption of phosphorus takes place by calcium ions under the condition of basic to neutral pH. Efficient adsorption through iron ions takes place at neutral to acidic medium under an aerobic environment. Aluminum ions are also responsible for the precipitation of phosphorus. Macrophytes in their growth period take up phosphorus in the range of 30–150 kg ha^{-1} $year^{-1}$ (Sundaravadivel and Vigneswaran 2001). In CWs, phosphorus is not removed completely, it is adsorbed only by the metal ions, bounded in the supportive media and taken up by macrophytes. CWs and their nutrients removal efficiency as reported in various studies worldwide are presented in Table 1. In this review-based study, we discuss appropriate environmental and operational parameters that are crucial for the sustainable working of CWs towards domestic wastewater treatment.

Environmental factors

pH

The pH of wastewater in CWs employs significant effects on numerous reactions and mechanisms along with biotic conversion, cation exchange and solvability of gases and solids (Niveditha 2019). The growth and development of macrophytes as well as action of nitrifying and heterotrophic bacteria also need optimum pH (near neutral) (Ilyas and van Hullebusch 2020). It pedals several biological processes and helps in the ionization of the compounds. The occurrence of macrophytes in wetland system regulates the pH (~ 7.5) and impacts the removal efficiency (Ilyas and van Hullebusch 2020). The water chemistry and other biotic processes are greatly affected by the pH of wastewater in the wetland system (Sun and Austin 2007). The optimal pH required for the *Nitrosomonas* varied from 7.9 to 8.2, for *Nitrobacter* ranged from 7.2 to 7.6 and denitrifying bacteria performed well at pH 6.5–7.5 (Rodriguez-Sanchez et al. 2014; Huang et al. 2019). It is reported that the pH of wastewater drops significantly through the nitrification process. Studies have shown that the highest activity of denitrifying bacteria was found at

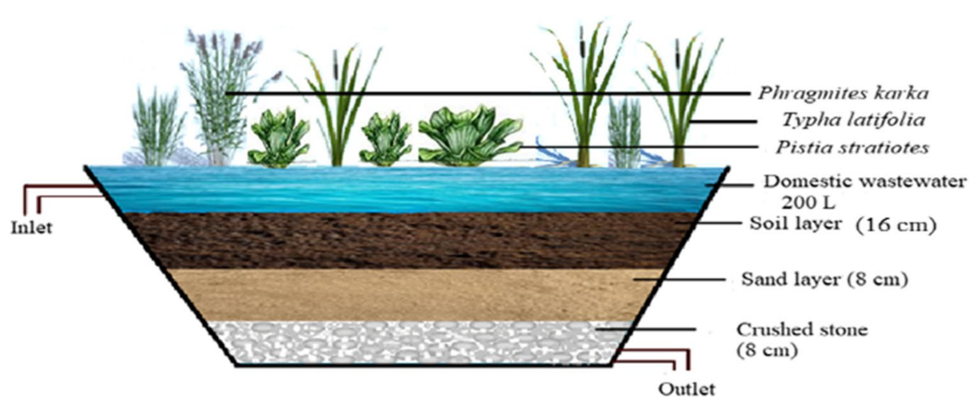

Fig. 1 A working model of CWM designed using emergent and floating macrophytes with different substrate materials

Table 1 CWs and their nutrients removal efficiency as reported in various studies in different regions of the world

Country	Wastewater type	Dimension L × W × D (m)	Macrophyte used	Removal Efficiency (%)				HLR (m^3/day)	HRT (day)	References
				NH_4^-N	NO_3^-N	TN	TP			
New Delhi, India	Municipal sludge and Tertiary	69 × 46 × 0.3	*Phragmites australis*	–	–	67	75	43.05	5.15	Ahmed et al. (2008)
Southern Spain	Municipal and storm-water	23.5 × 13.5 × 0.8	*Phragmites australis* *Typha spp.*	74	–	66	22	0.044	–	Ávila et al. (2013)
South Brazil	Municipal sewage	17 × 17 × 2.5	*Typha domingensis*			41	37	67.4	11.5	Benvenuti et al. (2018)
Marrakech-Morocco	Municipal	13 × 10 × 0.9	*Phragmites australis*	–	–	67	62	0.5 and 0.75	14	Elfanssi et al. (2018)
Heraklion, Greece	Domestic	8.4 × 5.4 × 0.45 m	*T. parviflora, J. acutus, S. perrenis, L. monopeta-lum*	0	16	22	13	0.053	3.48	Fountoulakis et al. (2017)
Heilongjiang, China	Rural sewage	14.0 × 1.2 × 0.6	*Hemerocallis lilioaspho-delus, Iris tectorum, Oxalis violacea, Sedum erythrostictum,* and *Hosta ensata*	70.98	–	–	36.48	0.025	24	Gao and Hu (2012)
Wuhan, China	Domestic	3 × 8 L × W	*Canna indica, Juncus effusus, and Scirpus validus*	79.2	–	57.7	82.8	0.24	1	He et al. (2018)
Peradeniya, Sri Lanka	Municipal and second-ary	25.0 × 1.0 × 0.6	*Scirpus grossus* *Typha angustifolia*	74.4	50.0	–	19.0	13	1.2	Jinadasa et al. (2006)
Zhejing, China	Lake water	20.0 × 1.5 × 1.0	*Typha angustifolia*	32.0	65.3	52.1	65.7	0.64	–	Li et al. (2008)
Santo Tomé, Argentina	Industrial and sewage	50 × 40 × 0.5	*Typha domingensis* *Eichhornia crassipes*	28	72	–	43	100	7–12	Maine et al. (2007)
Valencia, Spain	Lake water	715–9791 m^3	*Cattails* *Rushes*	78.07	58	52	65	11,232	–	Martin et al. (2013)
Nairobi city, Kenya	Municipal and second-ary	7.5 × 3.0 × 0.6	*Cyperus papyrus*	42.76	26.36	–	42.86	–		Mburu et al. (2013)
Dong Ha city, Vietnam	Sewage	4.44 m^2	*Canna indica* and *Colocasia esculenta*	84.2 65.0 82.0	–	82.0 74.0 82.0	1.3 15.0 23.0	100 200 150	2.65 1.32 1.76	Nguyen et al. (2018)
Haridwar, India	Urban Sewage	7.8 × 6.65 × 1.8	*Typha latifolia, Phrag-mites australis* and *Colocasia esculenta*	55.3	63.09	–	58.29	-	3	Rai et al. (2015)
Ontario, Canada	Municipal	11.8 × 8.6 × 1.2	*Typha latifolia*	98.2	–	98.8	–	16.62	–	Rozema et al. (2016)
Bosnia and Herzegovina	Domestic	20 m^2	*Typha latifolia and Phragmites australis*	84.52	–	57	69	9.89 to 2.51	5	Toromanovic et al. (2017)
Can Tho University, Vietnam	Municipal and second-ary	12 × 1.6 × 1.1	*Phragmites* *Vallatoria*	91 69 65 –	– – - –	84 61 62 16	99 98 85 72	0.031 0.062 0.0104 0.0146		Trang et al. (2010)

Table 1 (continued)

Country	Wastewater type	Dimension L × W × D (m)	Macrophyte used	Removal Efficiency (%)				HLR (m^3/day)	HRT (day)	References
				NH_4^-N	NO_3^-N	TN	TP			
Goa, India	Raw Sewage	CW 1—4 m^2	*Typha angustata*	21	–	15	34	150	1	Yadav et al. (2018)
		CW 2—1.88 m^2	*Canna indica*	52		50	58			
				58	–	35	–	225		
				82		47				
Waterloo, Canada	Municipal	2.4 × 0.4 × 0.2	*Carex aquatilis Schoenoplectus tabernaemontani*	97.8	–	89.2	-	27	2–3	Yates et al. (2016)
Chongqing, China	Raw Sewage	4 × 4 × 1.5	*Cyperus alternifolius*	76	–	65	65	150	5.25	Zhai et al. (2016)
		7.5 × 7 × 8		72		45	52			

NH_4-N Ammonium-Nitrogen, *NO_3-N* Nitrate-Nitrogen, *TN* total nitrogen, *TP* total phosphorus

a pH ranging between 7.0 and 7.5 (Kraiem et al. 2019; Sun et al. 2019; Fu et al. 2020; Wei et al. 2020). Methanogens are more active at a pH range of 6.5–7.5. It is also known that a certain alteration in pH can diminish the activity of methanogens in CWs ultimately resulting in the formation of odorous substances (Saeed and Sun 2012).

Temperature

The temperature has a significant impact on the removal of wastewater nutrients by promoting the growth of microorganisms that helps in the breakdown of contaminants (Wang et al. 2016). Microorganisms are the vital components for the elimination of nitrogen and phosphorus. The processes of ammonification, nitrification and denitrification are correlated with temperature; thus, affecting the elimination rate of total nitrogen (TN) (Zhang et al. 2018). Usually, in subsurface flow constructed wetlands (SSFCWs), the most favorable temperature range for nitrification is 16.5–32 °C and a decrease of 5 °C or increase above 40 °C strongly inhibits the action of nitrifying bacteria (Shi et al. 2018). The rate of nitrification inhibited greatly at a temperature lower than 10 °C and became severe at less than 6 °C because of the fall in the growth and developmental rate of microorganisms (Chang et al. 2012). On the contrary, the denitrification rate is also very poor at a temperature less than 5 °C and maximum at temperature ranging from 20 to 25 °C (Shi et al. 2018). Removal of orthophosphate (PO_4^-P) and total phosphorus (TP) have more dependency on temperature. A significant increase in temperature promotes polyphosphate-consuming microbes (Wang et al. 2016). To alleviate the adverse impact of temperature, traditional machinery of CWs requires certain modifications. For example, Wu et al. (2011) utilized mixtures of sand, pea gravel and washed gravel as the packed media in vertical flow constructed wetlands (VFCW), beneath 0.4 m layer of sawdust, known as an 'insulating layer' for the treatment of domestic wastewater. This modified system confirmed less decrease in removal efficiency of NH_4^-N by sustaining the higher temperature within the supportive material bed (above 6 °C) during the winter period. Seasons have major effects on the removal of wastewater nutrients. In the context of TN removal, summer has the lowest mean effluent TN concentrations, which might be due to the highest plant uptake. Seed and Sun (2012) described that the contribution of macrophytes for the removal of nitrogen is about 0.5–40%. Several reactions take place inside the wetland's supportive material, in which, breakdown of contaminants and microbial activity produces enough heat to save the surface layers from freezing. Earlier studies have reported that the removal efficiency of nitrogen dropped in winter as compared to summer in the Mediterranean climate in the vicinity of Marseille, France that is characterized by low annual rainfall during autumn and spring and a hot dry summer (Wang et al. 2012). This is because most of the macrophytes, such as *Typha orientalis, Phragmites australis, Nelumbo nucifera, Acorus calamus, Juncus effusus* and *Canna generalis* in winter went into dormancy ultimately reducing the growth rate (Fan et al. 2016). Furthermore, the activities and diversity of microorganisms may also be repressed (Wu et al. 2015). Tuncsiper (2007) reported 9% and 7% higher NO_3^-N and NH_4^-N removal respectively from tertiary treated wastewater throughout summer as compared to winter in Istanbul, Turkey. It was also reported (in the same study) that the VFCWs were inappropriate in winter as compared to horizontal flow constructed wetlands (HFCW).

Availability of dissolved oxygen (DO)

Availability of DO in wastewater is the principal environmental factor that pedals biodegradation of organics and nitrification rate. Zhang et al. (2010) evaluated a relative assessment among four HFCW, which was categorized as without and with macrophytes and aerated and non-aerated systems. The authors described higher TN removal efficiencies (mean 76.9–86.0%) and NH_4^-N (mean 72.3–89.1%) with macrophytes along with aeration. A different comparative study concerning with and without macrophytes with aerated and non-aerated system by Maltais-Landry et al. (2009), showed the complementary functions of macrophytes beside the artificial aeration, to smoothing TN elimination rates. The presence of macrophytes with aeration system improved TN removal efficiency up to 48.12%, as compared to without macrophytes and non-aerated system. Consequently, from these comparative studies with and without aerated CWs, the vital role of DO on biodegradation of organics and nitrogen is important. One significant feature of aeration is the diffusion rate of oxygen from the aerators, essential to sustain anaerobic and aerobic regions, predominantly when concurrent nitrification–denitrification is desirable. Removal of nitrogen based on DO present in the effluent may not always show the real depiction of the microbial reactions within the CWs units (Saeed et al. 2012) and also higher DO in the effluent of CWs systems does not essentially show aerobic situation. Aerobic and anaerobic regions are continuously coexisting within the CWs units (Bakhshoodeh et al. 2020).

Working/operational parameters

Effects of C/N ratios

It is reported that the C/N ratio between 2.5 and 5 provides the highest nitrogen removal efficiency (Chen et al. 2020). The authors also stated that the optimum value of C/N ratio for equilibrium between nitrification and the denitrification is 10**.** The rates of nitrification and denitrification get altered under a high C/N ratio, which results in low nitrogen removal. Overall, the removal of nitrogen is extremely reliant on the level of pollution (Saeed and Sun 2012). Suitable input mechanisms for carbon and nitrogen are obligatory to attain efficient nutrient removal**.** The carbon source is a critical controlling factor for denitrification, particularly when the C/N ratio is less than 3 (Stefanakis et al. 2014). Traditional microbiological pathways for the removal of nitrogen are usually inhibited due to the absence of organic carbon. Adding carbon sources in the wetland system might tackle this challenge and sustain the denitrification process (Songliu et al. 2009). Zhao et al. (2010) applied three different C/N ratios in VFCW viz. 2.5, 5 and 10 respectively, and found that the removal rate of TN was maximum at C/N ratio of 2.5. On the other hand, a study carried out by Chen et al. (2020) for HFCW revealed that the removal efficiency of TN was maximum at C/N ratios of 5 and 10. It is evident from the above-mentioned studies that C/N ratios differ among different CWs depending upon the design, category of wastewater, plantation and composition of contaminants to be removed. Therefore, it is challenging to establish recommendations on C/N ratio to achieve a higher denitrification rate. It may be an option to supply organic matter internally through the supportive material to facilitate the heterotrophic elimination of NO_3^-N. Saeed and Sun (2012) and Tee et al. (2012) have reported such types of alternatives in which they identified rice husk and organic wood-mulch as supportive substrate materials to smoothen the elimination of NO_3^-N from domestic sewage.

Selection of appropriate macrophytes and substrate materials

Macrophytic species exhibit a vital role in the performance of CWs. Predominantly, *Typha* spp. possess higher contaminant removal efficiency particularly for phosphorus (Kumar and Dutta 2019b). Four types of aquatic macrophytes are being used in CWs for the treatment of almost all types of wastewaters throughout the globe. These are emergent (e.g. *Typha* spp., *Phragmites* spp., *Scirpus* spp., *Juncus* spp. etc.), free-floating (*Pistia stratiotes, Eichhornia crassipes* etc.), floating leaved (*Nuphar luteum*, *Nymphaea odorata, Nelumbo* spp. etc.) and submerged macrophytes (*Potamogeton crispus, Ceratophyllum demersum, Hydrilla verticillata, Vallisneria natans* and *Myriophyllum verticillatum*) (Kumar and Dutta 2019a). Out of these, the emergent macrophytes are considered as most appropriate because of their higher contaminants' uptake capacity. Emergent macrophytes such as *Typha* spp. and *Phragmites* spp. are most frequently used in Asia and Europe for wastewater treatment. The compactness of macrophytes in a CW system powerfully distresses its hydrological stability, principally, by reducing flow velocity. The roles of aquatic macrophytes in the wastewater treatment are: (a) they alleviate substrate material (b) cut down water velocities, (c) permit suspended constituents to settle down, (d) provide litter after death and decay, (e) root organization and stems make available sites for microbial growth and attachment, (f) relocate gases amongst the atmosphere and the sediments from subsurface vegetation arrangements, produce oxygenated microsites within the substrate material and finally take up wastewater nutrients, organic matter and trace elements by incorporating them into their tissues.

The choice of supportive material depends on the hydraulic permeability and its ability to absorb wastewater contaminants. The consequences of reduced hydraulic conductivity are blockage of structures, reduction in the efficacy of the system, and slowing down of adsorption process ultimately affecting the long-term removal efficiency of CWs (Wang et al. 2010). Permeability of the supportive material affects the driving of water current within the CWs. Several studies have been carried out on choosing the right supportive material particularly for the removal of phosphorus (Wang et al. 2010; Saeed and Sun 2012; Chong et al. 2013; Wu et al. 2015). Most widely used substrate materials primarily comprise of industrial by-products, artificial media and natural materials, such as sand, clay, gravel, zeolite, activated carbon, slag, calcite, lightweight aggregates, fly ash, marble, limestone, vermiculite, bentonite, dolomite, shell, and wollastonite (Saeed and Sun 2012; Chong et al. 2013; Kumar and Dutta 2019b). From the above-mentioned studies, it is concluded that the supportive materials such as gravel, sand, peat and rock are less appropriate for long-term phosphorus removal as compared to industrial by-products and artificial material with high hydraulic permeability. It is reported by Vymazal (2007) that the vermiculite media has a significant effect on the removal of phosphorus (25.5% higher) as compared to the natural substrate by providing large adsorption sites in the form of vermiculite surfaces.

Suitable design and appropriate site selection for CWs

Sustainable designs of CWs are scientific attempts to simulate the function of natural wetlands. Researchers have focused on the evaluation of wetland procedures that can improve the water quality. Mitsch (1992) suggests following guiding principles for building an effective CW system: (a) design must be simple (b) it should require negligible maintenance (c) natural forces like gravity flow to be used (d) design must be following prevailing environmental conditions, not in contradiction of it (e) design should withstand extremes of meteorological conditions such as floods, droughts and storms (f) system should be in integration with the natural landscape of the site (g) design should avoid over-engineering with most simple rectangular compartments, inflexible arrangements and channels (h) as CWs do not essentially become practical immediately, should be given sufficient system time, and (i) the CWs system should be designed for function, not for form.

Suitable site selection can also save substantial expenditures. Selection of location should evaluate land use, accessibility of the land, landscape settings, ecological assets of the site, soil and the potential effects on living organisms. A well-suited site for designing a CW system generally follows following settings: (a) proximity near the wastewater source (b) does not encompass endangered or threatened species (c) situated with sufficient slope so that wastewater can move through gravity (d) situated above the water table, not in a flood-prone area (e) efficacy of CWs might be inadequate unless a sufficient HRT is not provided (f) largely adequate to assimilate existing necessities as well as future adjustments and modifications (g) having sufficiently compacted soil that can diminish leakage to groundwater.

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

The HLR and HRT are the leading aspects that are capable to alter the treatment performance of a CW system. HRT may be described as the span of the period through which the contaminant is in interaction with the supportive material and vegetation. Larger HLR facilitates the faster movement of the wastewater through supportive materials, and decreases retention time. Stefanakis and Tsihrintzis (2012) have conducted extensive research of 10 VFCW, treating synthetic wastewater for three years. In the experiment, they selected three different HLRs: 0.19, 0.26 and 0.44 m/day, respectively for first, second and third year separately. They demonstrated that for each HLR increase, the efficiency was also increased for nitrogen removal. This remarkable outcome shows the complete development of macrophytic species. One more long-term observation by Cui et al. (2010) was that when HLR increased from 7 to 21 cm/day, an insignificant reduction of TN and NH_4^-N was observed from domestic sewage in VFCWs. The removal efficiency decreased from 65 to 60% for NH_4^-N and 30% to 20% for TN. It is also reported that the HRT has a strong impact on the microbial population and their arrangement, biogeochemical processes, and the concentration of nutrients (Ranieri et al. 2013). HRT is recognized as a critical regulatory aspect for defining the pollutant removal efficiency (Stottmeister et al. 2003). A longer contact time permits wide interaction among pollutants and various components of the wetlands system. However, in case of less contact time, wastewater passes out faster through the outlet and eventually reduces the interaction time with microbes and root organization of the macrophytes. This results in a decrease in the removal performance (Tuncsiper et al. 2015). Longer HRTs characteristically need extended land area and greater investment. Consequently, future studies need to evaluate the impact of different HRTs on the performance of CWs.

The mode of influent feed and effluent recirculation

The choice of appropriate feed mode is essential because it enables the mixing of wastewater within the supportive material. Currently, various kinds of feed modes have been reported such as step, continuous, tidal flow, intermittent and batch feed to improve treatment efficiency. The effect of discontinuous loading (4 times a day) on VFCWs to enhance the biodegradation process for the removal of nitrogen from domestic sewage was studied by Laber et al. (1997). The notable outcome of this study was that the higher rate of denitrification (approximately 89%) could be achieved after the addition of external carbon. Conversely, when the continuous loading mode was applied then the rate of denitrification reduced significantly. The influence of continuous and intermittent feeding was also explored for superficial HFCW by Osorio and García (2007). The researchers demonstrated that the alternate feeding mode had upgraded the removal efficiency of NH_4^-N, as compared to continuous feeding mode. Due to this improved efficacy, the researchers made two probable explanations, first macrophytic species make available DO at greater proportion and second, allied flushes during intermittent loading prompt more turbulence within the supportive material, that's why wastewater moves into anaerobic and aerobic zones. Recently, extensive modification in feeding mode has been applied to simplify the removal of organics and nitrogen through the effective use of aerobic environments within the supportive material. For such modifications, the tidal flow VFCWs is a suitable example (Wu et al. 2011).

In the CWs system, the recirculation of wastewater increases the removal of nitrogen predominantly when the settings become DO deficient (Lavrova and Koumanova 2010). It also advances the rate of denitrification because the contact time between effluent and biofilms get increased with the supply of additional organics (Zhao et al. 2004). Several studies elaborate on the various recirculation ratios for attaining advanced nitrogen removal. Ayaz et al. (2012) designed a hybrid system for the treatment of domestic wastewater. They recirculated wastewater at a ratio of 1:1–2:1 and found a significant increase in the removal efficiencies of TN (approximately 66%) as compared to without recirculation (19–55%). Lavrova and Koumanova (2010) worked on effluent recirculation in VFCW systems and got better results. Principally the effluent recirculation mode enhances removal efficiency by diluting received wastewater. Recently a modified form of VFCW named as recirculating vertical flow wetland (RVFW) was developed by Sklarz et al. (2009), which depends on continuous effluent recirculation to the root region until the optimum quality of wastewater is achieved. This new system reduced 31 mg/L nitrogen concentration. The use of this RVFW system is more advantageous as (a) it permits widespread aeration and dilutes the raw wastewater (b) encourages the upkeep of the committed microbial biomass by saturation of supportive material. Nevertheless, the application of this system is also limited in the area where the loading rate is very high and continuous and requires high energy input that may increase the operational cost.

Suitable plant harvesting strategies

The aboveground macrophyte biomass is typically harvested to eliminate wastewater contaminants absorbed in vegetative parts. Periodic harvesting of macrophytic biomass is the best alternative for the management of CWs in the removal of contaminants (Jinadasa et al. 2008; Ranieri and Gikas 2014). The optimum time for harvesting is determined by the interaction between nutrient concentrations and total biomass. Harvesting in the summer season can delay the successive growth of macrophytes and disturb nutrient movement within the macrophytes with radial oxygen loss (ROL) (Wang et al. 2014). They described that the late autumn harvesting had an adverse effect on the removal of ammonia (NH_4^+-N) with microbial richness and action, and lesser plant radial oxygen loss. Further, the biological significance and utilities of macrophytes are dissimilar among winter and summer seasons. However, there is uncertainty about the impact of removal performance and microbial richness due to summer harvesting. Harvesting of macrophytes from CWs can also enhance the removal of nitrogen (Jinadasa et al. 2008). Harvesting is responsible for the elimination of nitrogen in the range of 0.27–0.68 g/m^2/day reported by Kadlec et al. (2000). In another study by Borin et al. (2001) 120 kg/ha of nitrogen removal was attained through harvesting in summer. It is mostly in the summer season, macrophytes proliferate vigorously and take part in the uptake of nutrients as compared to winter season (Greenway 2005).

Artificial aeration and bioaugmentation of microorganisms

Aeration is applied due to its capability to improve the removal efficiency of nutrients as compared to non-aerated systems (Wang et al. 2015). It appeared to improve bacterial nitrification by creating favorable conditions for their action and removal of phosphorus by enhancing oxidation of Fe^{2+} to Fe^{3+} (Li et al. 2014). Moreover, aeration also enhances the degree of resistance against the inconsistent influent loads and inhibits blockage by increasing mineralization of solids, consequently increasing the working duration of CWs (Butterworth et al. 2016). A study carried out by Wu et al. (2016) revealed that the intermittent aeration with sludge-ceramsite substrate material considerably enhanced the removal performance of nitrogen (NH_4^+-N—98.9% and TN—85.8%). Intermittent aeration is more efficient than continuous aeration because intermittent aeration has more ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Removal of nitrogenous compounds has been validated in numerous studies by introducing especially adapted bacterial populations (Wang et al. 2011) or little quantity of a bacterial community suspension (Zaytsev et al. 2011) with anaerobic ammonium oxidizing (anammox) bacteria and modified soil (Zou et al. 2009). The introduction of the bacterial population reformed the soil microbial structure by altering the species equitability and developing different microbial population equilibrium in situ (Zhao et al. 2016). Microorganisms with fewer contaminant wetlands may accumulate more phosphorus than contaminant rich conditions. Hou et al. (2011) reported that the bacterial population such as *Paenibacillus* sp. possesses the highest rate of TP removal. Bioaugmenting microbial population in CWs could provide a cost-effective and possible choice in colder regions. There is a lack of information on the feasibility and treatment efficiency of bioaugmented CWs in colder regions with specific perseverance (Wang et al. 2012). Several microbial communities identified in CWs are presented in Table 2.

Reuse of treated wastewater

Treated wastewater reuse for non-potable purposes is a possible solution to augment water supply in water-scarce areas. It can be used to tackle water stress and to improve the groundwater table through recharge after suitable quality standards have been achieved. The treated wastewater may be used for agriculture and gardening to reduce the pressure on natural water resources (Declercq et al. 2020). It can also be discharged in freshwater bodies to support aquatic life and maintain the base flow. With these, treated wastewater

Table 2 The cumulative effect of macrophytes and microorganisms on the removal efficiency of nutrients in CWs as reported in various studies

Country	Wastewater	Macrophytes	Microorganisms reported	Removal efficiency (%)		References
				NH_4^+-N	PO_4^{2}-P	
USA	Synthetic	*Schoenoplectus tabernaemontani*	α-Proteobacteria (about 48–60%) Actinobacteria and Firmicutes	NA	79	Ahn et al. (2007)
China	Synthetic		Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Chlorobi, Chloroflexi, Firmicutes, Gemmatimonadetes, Hydrogenedentes, Ignavibacteriae, Nitrospirae, Parcubacteria, Planctomycetes, Proteobacteria and Verrucomicrobia	NA	NA	Li et al. (2019)
Colombia	Domestic	*Cyperus articulatus* and *Thalia geniculata*	180 (65 anaerobic and 115 aerobic) heterotrophic bacteria	83 83	74 74	Llanos-Lizcano et al. (2019)
USA	Run-off	NA	α-Proteobacteria was the most dominant class, followed by γ-Proteobacteria and β-Proteobacteria	NA	NA	Peralta et al. (2013)
China	Sewage	*Kandelia candel*	Vibrio, Candidatus Competibacter, Denitratisoma, Nitrospira, Thauera, Nitrosomonas, Planctomyces, Marinobacterium, Magnetospira, and Dechloromonas	74.9	NA	Fu et al. (2020)
Denmark	Domestic	*NA*	*Acinetobacter* sp. (γ-Proteobacteria), *Arthrobacter* sp., *Flavobacterium* sp., *Thauera terpenica, Xanthomonas* sp., *Dokdonella* sp., *Rhodanobacter* sp. and *Stenotrophomonas* sp.	60	NA	Adrados et al. (2014)
China	Swine	*Myriophyllum aquaticum*	*Firmicutes, Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Acidobacteria, Cyanobacteria, Planctomycetes, Verrucomicrobia and Deffeibacteres*	92.34	NA	Xu et al. (2020)

NA not available

also supports ecological services such as supporting valuable wildlife, fisheries, and promoting recreational uses (Kumar and Dutta 2019b).

Economic feasibility of CWs

CWs have emerged as suitable alternatives to traditional wastewater treatment technologies primarily for decentralized locations and remote areas (Kumar and Dutta 2019b). Their excellent capabilities of pollutant removal, coupled with eco-friendly design and materials reduce the operational costs substantially compared to oxidation and facultative ponds (Stefanakis 2020). They depend entirely on natural processes under controlled conditions, require low or no energy inputs, and employ gravity flow with low operational and maintenance costs. Their reliable efficiency and ecological benefits such as groundwater recharge, valuable wildlife habitat, carbon sequestration, aquaculture, fisheries, silt capture, flood control and recreational uses make them cost-effective to treat domestic wastewater (He et al. 2018) Nearly, most identified wastewater treatment technologies have relied on processes, such as filtration, sedimentation, biological activity etc. They are often designed with complex structures and consume much energy due to their mechanical equipment and operations (Stefanakis 2020). Several other traditional wastewater treatment technologies such as activated sludge, fluidized aerobic bed reactors (FAB), upflow anaerobic sludge blanket (UASB) and membrane bioreactors are also applied effectively for the treatment of wastewater. Due to the high cost involved in the maintenance and operation, the long-term application of such technologies is not feasible on field-scale or for decentralized locations (Zhang et al. 2020).

Future concerns and challenges

The design of CWs requires a large land area, and their performance is significantly affected by seasonal changes (Stefanakis 2020). Therefore, a suitable improvement in design could lessen the total land requirements. The selection of appropriate substrate materials, macrophytic species, and HLR and HRT optimization are vital for maximum removal efficacies (Kumar and Dutta 2019b). Suitable macrophyte harvest methods are also crucial because they leave nutrients and other pollutants into the respective water bodies after death and decay (Bhat et al. 2020). The future strategies should develop new techniques with enhanced treatment capabilities that could be accomplished by external carbon supply, novel substrate materials, step feeding, tidal action, and baffled flow systems. For the efficient working of field-scale CWs, the ongoing studies must be focused on inclusive assessment and fate of new substrate materials and macrophytes in real environments, optimization of several environmental and operational conditions (Kumar et al. 2020). The importance of bacterial populations must also be assessed while studying the interaction with macrophytes and substrate material (Batool and Saleh 2020).

Conclusion

CWs are known as sustainable, cost-effective, and environmental-friendly technology for the treatment of domestic wastewater, especially in decentralized systems where conventional treatment systems are not feasible. The emphasis of this study is to explain the suitable environmental and operational parameters that could enhance the effectiveness of wastewater treatment in a CW system. The pH, DO and temperature are crucial in treating domestic wastewater. Macrophytes and substrate material play an essential role in the removal of nutrients. Usually, removal of phosphorus proceeds mainly via adsorption, sedimentation, cation exchange, precipitation and uptake by macrophytes. Removal of nitrogen occurs mainly by microbial community and uptake through macrophytes. Therefore, the selection of appropriate plant species and supportive material is necessary. Removal efficiency has been improved significantly by recirculation of effluent, extra carbon and artificial aeration (intermittent). Augmentation of microbial communities is essential in terms of working in colder climates. Various other operating conditions such as suitable design and type of CWs, C/N ratios, mode of effluent HLR, HRT, availability of carbon source and plant harvest strategies are crucial to attaining the feasible treatment performance for enhanced sustainability.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest with respect to authorship, research and publication of this paper.

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Cleaning the River Ganga: Impact of lockdown on water quality and future implications on river rejuvenation strategies

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HIGHLIGHTS

- Signs of rejuvenation and significant improvement on many parameters in Ganga River, following nationwide lockdown due to coronavirus pandemic
- Lockdown period coincides excess rainfall (60 percent above normal), reduced irrigation and power demands in the basin resulting in increased storages and more flow in the river improving the quality
- Increasing trends of dissolved oxygen (DO) and decreasing trends of biological oxygen demand (BOD) and nitrate (NO3-) concentration
- River becomes fit for drinking (Class A) in the upper stretches and for outdoor bathing (Class B) in the middle and lower stretches

GRAPHICAL ABSTRACT

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ABSTRACT

Clean rivers and healthy aquatic life symbolize that the ecosystem is functioning well. The Ganga River has shown signs of rejuvenation and a significant improvement on many parameters, following the eight-week nationwide lockdown due to coronavirus pandemic. Since industrial units and commercial establishments were closed, water was not being lifted by them with a negligible discharge of industrial wastewater. It was observed that during the lockdown period most of the districts falling under the Ganga basin observed 60% excess rainfall than the normal, which led to increased discharge in the river, further contributing towards the dilution of pollutants. Further, data analysis of live storages in the Ganga Basin revealed that the storage during the beginning of the third phase of lockdown was almost double than the storage during the same period the previous year. Analysis of the storage data of the last ten years revealed that the storage till May 6, 2020 was 82.83% more than the average of the previous ten years, which meant that more water was available for the river during the lockdown period. The impact could be seen in terms of increased dissolved oxygen (DO) and reduced biological oxygen demand (BOD), Faecal coliform, Total coliform and nitrate (NO_3-) concentration. A declining trend in nitrate concentration was observed in most of the locations due to limited industrial activities and reduction in agricultural run-off due to harvesting season. The gradual transformation in the quality of the water has given a sign of optimism from the point of restoration. Yet, it is believed that this improvement in water quality is 'short-lived' and quality would deteriorate once the normal industrial activities are resumed, indicating a strong influence of untreated commercial–industrial wastewater. The paper concludes that the river can be rejuvenated if issues of wastewater and adequate flow releases are addressed.

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REVIEW

Recent advances in satellite mapping of global air quality: evidences during COVID-19 pandemic

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Abstract
There was a significant decline in air pollution in different parts of the world due to enforcement of lockdown by many countries to check the spread of the coronavirus (COVID-19) pandemic. In particular, commercial and industrial activities had been limited globally with restricted air and surface traffic movements in response to social distancing and isolation. Both satellite remote sensing and ground-based monitoring were used to measure the change in the air quality. There was momentous decline in the averaged concentrations of nitrogen dioxide (NO_2), carbon dioxide (CO_2), sulphur dioxide (SO_2), methane (CH_4) and aerosols. Many cities across India, China and several major cities in Europe observed strong reductions in nitrogen dioxide levels dropping by around 40–50% owing to lockdowns. Similarly, concentrations of SO_2 in polluted areas in India, especially around large coal-fired power plants and industrial areas decreased by around 40% as evidenced by the comparative satellite mapping during April 2019 and April 2020. Recent advances in sensors on board various satellites played a significant role in real-time monitoring of emission regimes over various parts of the world. The satellite data is relying upon single scene profusion for real-time air quality measurements, and also using averaged dataset over certain time-period. The daily global-scale remote sensing data of NO_2, as measured through the Copernicus Sentinel-5 Precursor Tropospheric Monitoring Instrument (S5p/TROPOMI) of European Space Agency (ESA), indicated exceptional decreases in tropospheric NO_2 pollution in urban areas. Similarly, Greenhouse gases Observing Satellite (GOSAT) of Japan Aerospace Exploration Agency, with a repeat cycle of three days helped in assessing the sources and sinks of CO_2 and CH_4 on a sub-continental scale.

Keywords Air quality · Aerosols · Satellite mapping · Copernicus Sentinel-5P · COVID-19

Introduction

Land use changes, anthropogenic emissions from transport and industries and climate variability deeply affect the environmental quality globally (Stavrakou et al. 2019). In the context of the worldwide lockdown enforced due to the COVID-19 pandemic, there is an increased interest in studying changing air quality through satellite remote sensing (Collivignarelli et al. 2020; Dantas et al. 2020; Li et al. 2020; Nakada and Urban 2020; Sharma et al. 2020). Lockdowns were imposed in several countries to impede the progress of the spread of the coronavirus pandemic within the communities. This resulted in cleaner air quality over the US, China, India and Europe. The concentration of pollutants markedly decreased over urban areas, with varying differences among primary and secondary pollutants (Bao and Zhang 2020; Huang et al. 2020; Liu et al. 2020; He et al. 2020). This was mainly achieved due to decrease in traffic and industrial activities. Satellite remote sensing is now using multi-platform system and new algorithm in the processing chain that enables better resolution of emission inventories. Significant decrease in NO_2, CO_2, SO_2, CH_4 and aerosols levels have been observed globally during the lockdown period. Real-time observations of background air quality precisely measure 'hotspots of air pollution' through the Copernicus Sentinel-5 Precursor Tropospheric Monitoring Instrument

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ORIGINAL PAPER

Impact of nutrient enrichment on habitat heterogeneity and species richness of aquatic macrophytes: evidence from freshwater tropical lakes of Central Ganga Plain, India

D. Dubey[1] · S. Kumar[1] · V. Dutta[1]

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Abstract

Lakes respond to nutrient enrichment by varying degrees, and it is important to understand how species richness of macrophytes is affected by anthropogenic disturbances in urban and rural catchments. Trophic state and macrophytic diversity of three tropical lakes exposed to different anthropogenic stresses have been studied in this paper. It is observed that changes in nutrient loading and anthropogenic interference result in changes in the community structure of macrophytes. Trophic state of lakes was measured using three main parameters viz. total phosphorous (TP), chlorophyll a and Secchi depth. To quantify the impact of pollution on macrophytic diversity, diversity indices such as species richness, Shannon–Wiener index and importance value index have been estimated. With increasing TP, a significant decline in the species richness of submerged macrophytes was observed, while species richness of floating macrophytes increased. The two lakes in urban areas were found to be in mesotrophic state, while the one in rural area with agricultural catchment was found to be in oligotrophic condition. It was observed that lake's trophic state along with anthropogenic disturbances affected the dominance, composition and diversity of various macrophytes. Low TP favors abundance of submerged and free-floating macrophytes which increases habitat heterogeneity and species richness. With increasing nutrient loading, a major shift has been observed in the trophic state and macrophytic diversity. Species evenness was maximum in mesotrophic lake, main reason being the excessive growth of free-floating invasive species causing biotic homogenization, mainly attributed to high TP loadings.

Keywords Freshwater lakes · Water quality · Trophic state · Species richness · Species evenness · Eutrophication

Introduction

Freshwater lakes are among the most valuable natural resources as they play an important role in maintaining micro-climate by modulating atmospheric temperature, support aquatic flora and fauna and help in groundwater recharge thereby improving local environmental conditions (Rudolf et al. 2002; Obst 2003; Chen et al. 2010a, b; Bai et al. 2013; Zhang et al. 2014; Pittock et al. 2018; Zadereev et al. 2020). Freshwater lakes respond very quickly even to a small change in the magnitude and intensity of various biotic and abiotic factors (Wetzel 2001). Due to various anthropogenic disturbances, the unique ecological functions and diverse economical values of freshwater lakes are decreasing at a faster rate (William and James 2007; Liu et al. 2012).

Physicochemical integrity of lake water and sediments significantly determine diversity of aquatic flora and fauna. Various spatiotemporal factors such as catchment's land use patterns, nutrient concentration, water inflow and organic matter influence the macrophytic communities within their lake ecosystem (Sillanpaa et al. 2004; Thomaz and Cunha 2010). Importance of macrophytes in littoral zone is far reaching as they contribute to the structure, function and diversity of the ecosystem which help in nutrient cycle, support food chain along with providing habitat for invertebrates and fishes (Carpenter and Lodge 1986; Ozimek et al. 1990; Madsen et al. 2001; Rinke et al. 2019).

Increasing population and related unplanned urbanization, as well as environmental stressors like eutrophication, encroachments, siltation and invasion of exotic species,

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REVIEW

Practice of wastewater irrigation and its impacts on human health and environment: a state of the art

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Abstract
The practice of wastewater irrigation lessens the pressure on the aquatic environment by minimizing the use of freshwater resources. However, this may lead to significant damage to the human health and environments. Recycled wastewater possesses a substantial amount of nutrients that act as fertilizers for crops and facilitate the metabolic action of microorganisms. The major advantages of wastewater irrigation are increased agricultural production, nutrient recycling, reduced stress on freshwater, economical support and provision of livelihoods for farmers. However, several harmful impacts of wastewater irrigation are also prominent due to inappropriate wastewater management and irrigation practices. These include severe hazards to farmer's health, contamination of agricultural land and crops with toxic metals, chemical compounds, salts and microbial pathogens. In addition, long-term irrigation using wastewater can significantly affect the groundwater through leakage of salty and toxic metal-rich wastewater making it unfit for human consumption. Wastewater irrigation may also alter the physicochemical properties and microbiota of soil, which in turn can disturb land fertility and crop productivity. Several factors need to be considered while using treated or partially treated wastewater for irrigation such as diversity and type of pollutants, available nutrients, pathogenic microorganisms and soil salinity. In this review paper, we assess the impact of wastewater irrigation on humans as well as on the environment based on available case studies globally, outline current use of wastewater for irrigation of agricultural crops such as cereals, vegetables, fodder crops, including agroforestry and discuss suitable management practices of wastewater reuse for irrigation.

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Chapter 11
Efficiency of Constructed Wetland Microcosms (CWMs) for the Treatment of Domestic Wastewater Using Aquatic Macrophytes

Saroj Kumar and Venkatesh Dutta

Contents

Abstract Constructed wetland microcosms (CWMs) are engineered wastewater treatment systems that are designed to treat wastewater from small communities, involving aquatic plants, a variety of substrate materials, soils and their associated microbial fauna. CWMs are considered as promising ecological technology that requires low or no energy input, low operational cost and provides more benefits and better alternative to conventional wastewater treatment systems. In CWMs dissolved oxygen (DO), pH and temperature are controlled to achieve the desirable treatment efficiency. Several other components such as plant, substrate, water depth, hydraulic loading rates (HLRs) and hydraulic retention time (HRT) are also critical to establishing viable CWMs for the better performance. The literature on CWMs suggests excellent nutrient removal performances which are achieved with low and

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Chapter 14
Microbial Communities in Constructed Wetland Microcosms and Their Role in Treatment of Domestic Wastewater

Saroj Kumar, Bhanu Pratap, Divya Dubey, and Venkatesh Dutta

Abstract Microbial biomass is the main reducer for majority of organics and nutrients. The aerobic region of constructed wetland microcosms (CWMs) is majorly characterized by presence of *Nitrosomonas* and *Pseudomonas spp.* The diversity of ammonia-oxidizers mainly *Nitrosospira sp.* is higher in CWMs designed to treat domestic wastewater as compared to other bacteria studied. The activity of enzymes within CWMs is a key indicator towards role of microbial community. Rhizospheric region has diverse elements that comprises minerals, sugars, vitamins, organic acids, polysaccharides, phenol and various other organic materials that encourages the microbial groups to degrade wastewater pollutants. The presence of macrophytes has significant effects on microbial richness and community structure. The root exudates liberated by macrophytes are also able to alter the richness and diversity of the microbial population. The decomposition rates of microbes become slow as temperatures drop, which can be optimized by increasing the size of wetlands to accomplish the slower reaction rates. The pH of wastewater has also a strong effect on various microbially mediated reactions and processes. Temperature, hydrologic conditions, macrophytic diversity/richness and biotic succession strongly impact the microbial community structure. A little alteration in the diversity or community structure of the microorganisms directly affects the treatment performance of CWMs.

Keywords Microbial diversity/richness · Constructed wetland microcosms · Removal efficiency · Enzyme activity · Macrophytes

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