Ph.D. thesis entitled

Vertical Up-flow Constructed Wetlands to Improve Secondary Treated Wastewater: Microbiological Contaminants Removal

Submitted by

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Under the Supervision of

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CERTIFICATE

This is to certify that the thesis entitled "**Vertical Up-flow Constructed Wetlands to Improve Secondary Treated Wastewater:** ***Microbiological Contaminants Removal***" is being submitted by **Ms. Gargi Sharma (ID. NO: 2009RCE101)** to the Malaviya National Institute of Technology, Jaipur for the award of the degree of **Doctorate of Philosophy** is a bonafide record of original research work carried out by her. She has worked under our guidance and supervision and has fulfilled the requirement for the submission of this thesis, which has reached the requisite standards.

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Abstract

Constructed wetlands (CWs) are becoming popular decentralized wastewater treatment for smaller communities where land is easily available and frequent power tripping prohibits the advocacy of highly mechanized plants. Moreover, CWs have emerged as a viable alternative for treatment of physico-chemical parameters as well as their efficacy for microbial contaminants reduction from wastewater has drawn interest of researchers in recent past. Therefore, the present study was conducted to investigate the performance efficiency of constructed wetland treatment system during post treatment of secondary treated effluent, where the receiving water bodies such as rivers in India are found to be heavily polluted due to discharge of inadequately treated wastewater.

The research was carried out to improve the secondary treated wastewater in terms of both, indicator microbial species (*Total coliforms* and *Fecal streptococci*) and pathogenic specie (*Salmonella typhi*). Within this aim initially three vertical subsurface up-flow constructed wetlands were established with different size gravels layers - two of them were planted with *Phragmites australis* (UN-ph) and *Canna indica* (UN-cn) and one was unplanted or control unit (UN-ct). The performance efficiencies of all the three wetland units were evaluated for microbial contaminants removal along with their physico-chemical parameter analysis viz. pH, dissolved oxygen (DO), chemical oxygen demand (COD), Total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH_3-N) and nitrate nitrogen (NO_3^--N). The physico-chemical parameters were studied as a background analysis whereas the focus was maintained on microbial contaminants removal.

The statistical analysis of the experimental results showed that all the wetland configurations were effectively able to treat low strength secondary treated wastewater. Moreover, the study highlighted the comparative and significant suitability of *Canna indica* plantation over the *Phragmites australis* for removal of indicator microbial species results under the prevailing semi-arid climatic conditions at the experimental site which was located in MNIT campus at Jaipur, Rajasthan, India. The mean observed log removals of indicator species *Total coliform* and *Fecal streptococci* from the unit UN-cn were 1.87 and 1.82 log unit, respectively. Whereas

the corresponding removals from the unit UN-ph were 1.01 and 1.01 log unit, respectively, and from the unit UN-ct were 1.24 and 1.03 log unit, respectively. Since the effective treatment was observed from all CWs, the higher removal from the unit UN-cn was related to its fibrous rooting system which helps to maintain higher aerobicity throughout the system and that was also supported by the highest dissolved oxygen content in this unit.

On the basis of preliminary results of the first three units, further modifications in the wetland design were carried out in order to further reduce the coliform numbers within the WHO standard limit of 1000MPN/100ml for safe disposal into water bodies. Such modifications led to the development of the fourth unit UN-cn-sd which was planted with same *Canna spp.* with different media configuration – a 15cm soil layer was included along with two different sized gravels. Its configuration was based on the previous *Canna spp.* planted unit (UN-cn). Finally the performances of all the four different vertical up-flow CW units were evaluated together by analyzing their experimental results, and comparative analyses are presented in the thesis. The results are presented in such a way to analyze each physico-chemical and microbial parameter in different figures separately. Each figure shows comparative analysis of all units for a particular parameter.

The results show that the increased removal of indicator microbial species was achieved from the modified unit UN-cn-sd with mean observed log removals of 3.01 and 2.80 log unit for *Total coliform* and *Fecal streptococci*, respectively. Thus, the increased physical process and aerobic metabolism in the unit UN-cn-sd, due to the presence of sand and canna rooting pattern, was found to be more suitable treatment pathway in the proposed research. However, the removal pattern of pathogenic specie *Salmonella typhi* was found to be following a different pathway than indicator species. The removal order of *Salmonella typhi* from all the units was same as was the order observed for COD removal. So, the removal of *Salmonella typhi* was related with the competition occurring between the nitrifiers and decomposers for the carbon source. Ultimately, the performance of the vertical flow system with configurations based on the fourth unit UN-cn-sd, was deemed to be suitable technology for improving the quality of secondary treated wastewater.

Furthermore, the study was carried out to give a detailed knowledge about the microbial (bacterial) assemblages and attempted to suggest better understanding about functioning of CWs. Such characterization of bacterial assemblages helps to correlate the performance efficiency of the treatment units. The culture dependent method was used to isolate the species from plant roots and substrates of constructed wetland units and the isolated species were identified through biochemical testing. The retrieved species were affiliated with Proteobacteria, Firmicutes and Actinobacteria. The analysis of bacterial assemblages of all constructed wetland units was defined in terms of percentage-richness distribution and diversity. Analysis revealed that the most predominant phylum was Proteobacteria followed by Firmicutes and then Actinobacteria. Also the diversity was affected by the number of representative species of Proteobacteria. High diversity in the unit with sand layer indicates that increased surface area and increased development of biofilm supports the more diverse bacterial assembly in the wetland system and contributes to better performance efficiency.

Hence, the proposed study helps in determining the role of wetland design, bedding pattern and plant species in effluent treatment in terms of microbial reduction. Findings of this study can be incorporated as a significant and valuable addition to the existing knowledge and could be taken into consideration by institutions wherever tertiary treatment steps after sewage treatment plants (STP) are of concern.

Abbreviations

(A)	Actinobacteria
ASP	Activated Sludge Process
APHA	American Public Health Association
NH_3-N	Ammonia Nitrogen
ANAMMOX	Anaerobic Ammonium Oxidation
A_2O	Anoxic, Anaerobic and Oxic Chambers
AMATS	Aquatic Macrophyte-based Systems
BOD	Biological Oxygen Demand
CPCB	Central Pollution Control Board
CSE	Centre for Science and Environment
COD	Chemical Oxygen Demand
CFU	Coliform Unit
CFU	Colony Forming Unit
CW	Constructed Wetland
UN-cn	Constructed wetland unit planted with *Canna indica*
UN-cn-sd	Constructed wetland unit planted with *Canna indica* including sand
UN-ph	Constructed wetland unit planted with *Phragmites australis*
DO	Dissolved Oxygen
EC	Electrical Conductivity
E.coli	*Escherichia coli*
FS	*Fecal streptococci*
(F)	Firmicutes
Q	Flow
Gr	Gravel
HFCW	Horizontal Flow Constructed Wetland
HRT	Hydraulic Retention Time
IVCW	Integrated Vertical Flow Constructed Wetland
M	Meter

MR-VP	Methyl Red-Voges-Proskauer Test
mg/l	Milligram/litre
mm	Millimetre
MLD	Million Litre per day
MPN	Most Probable Number
NRCD	National River Conservation Directorate
$No_3^{-}N$	Nitrate Nitrogen
NA	Nutrient Agar
P	Phosphorus
(P)	Proteobacteria
S.typhi	Salmonella typhi
STP	Sewage Treatment Plant
SSF-CW	Sub Surface Flow Constructed Wetland
TC	Total coliform
TDS	Total Dissolved Solids
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TS	Total Solids
TSS	Total Suspended Solids
THM	Tri-Halo-Methane
TSI	Triple Sugar Iron
UV	Ultra Violet
UN-ct	Unplanted or Control Unit
VUF	Vertical Up Flow
VUFCW	Vertical Up Flow Constructed Wetland
V	Volume
WW	Wastewater
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate Agar
YEAMA	Yeast Extract Mannitol Agar

Table of Contents

List of Tables

List of Figures

Chapter 1
Introduction

1.1 Context and Background

Developing countries today are facing grave environmental problems due to the fast depleting natural resources of freshwater, which already represents a very small fraction (~3%) of the world's total water resources thus threatening the very existence of most of the ecosystems (Gleick et al., 2004). Analysis of water resources indicates that in the coming years, as cities and population grow leading to increasing water demand for agriculture, industry and household, it will be a huge problem to deal with fresh water availability worldwide. The issue of water scarcity in India is expected to get worse as the population is increasing at the rate of 1.9% per year and is expected to reach 1.6 billion by 2050. With increasing population, the total per capita availability of fresh water is expected to reduce to 1341 m^3 in 2025 and 1140 m^3 in 2050 (Kaur et al., 2012). This water demand-supply gap is continuously increasing and hampering the development process in many sectors. In order to bridge the gap between increasing demand against the depleting availability of fresh water, many advanced wastewater treatment technologies are being conceived and employed. Employment and empowerment of treatment technologies promote water reclamation and reuse with the goal of environmental protection. To further reduce the demand and supply gap, reuse of treated effluents for non-potable purposes could be promoted as a huge volume of wastewater is being generated and treated. But there are many challenges in prompting the reuse of treated effluents as most of the sewage treatment plants are designed to meet the discharge standards majorly in terms of removing the organic loading only and minor focus is on removal of microbes. Regulation of the wastewater treatment plants has been possible by imposing strict legislations and establishing high standards on discharge of treated water quality by many government authorities worldwide.

1.2 Conceptual and theoretical framework

The conceptual framework shown in Figure 1.1 represents the background of origin of the research problem and suggests information for formulation of this research approach. The highlighted area in this figure defines the direct approach while rest suggests the alternatives for the corresponding issues. The figure clearly indicates that the research problem of the proposed study originated due to the scarcity of fresh water availability which is a result of increasing population. As per the discouraging estimates of the present scenario as well as for years 2025 and 2050, there would be high need to go for the alternatives of freshwater. There can be three ways to overcome this problem: demand reduction, technology advancement and reuse of wastewater.

Appropriate water reclamation and reuse technologies have been adopted as the most feasible alternative to overcome the problems of freshwater availability by many cities around the world. But an estimates suggests that the observed treatment capacity is only 30% of total generated sewage (Kaur et al., 2012). The health risks exist with (secondary) treated effluent due to microbial contaminants and others such as pesticides, antibiotics and metals etc., as shown in conceptual framework. This research, is focused on removal of microbial contaminants from secondary treated wastewater and details will be discussed in following subheadings. Thus, we can clearly see the approach (highlighted area in figure 1.1) of the present research study through viewing the conceptual framework.

The theoretical framework shown in Figure 1.2 represents the target of the proposed research which follows treatment technologies and focused contaminants. Although the framework highlights the main objective of the research, it also includes the required rest of the knowledge as a background information. Thus, the theoretical framework given below provides insights about the clear goal of the study which is to reduce the coliform number using constructed wetland when they are consistently present from raw sewage even after secondary treatment.

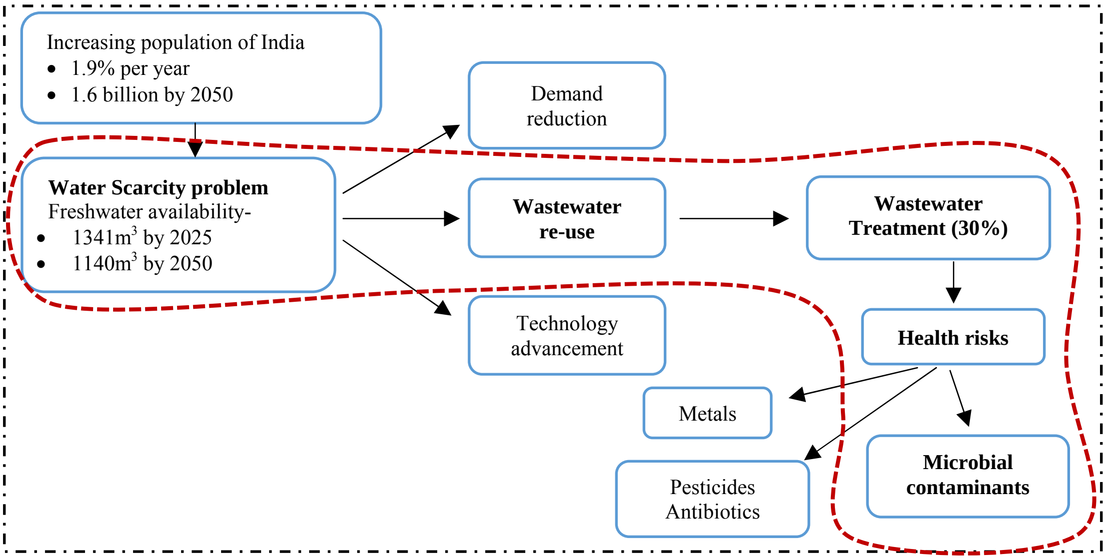

Fig. 1.1. Conceptual framework – Approach towards the research problem

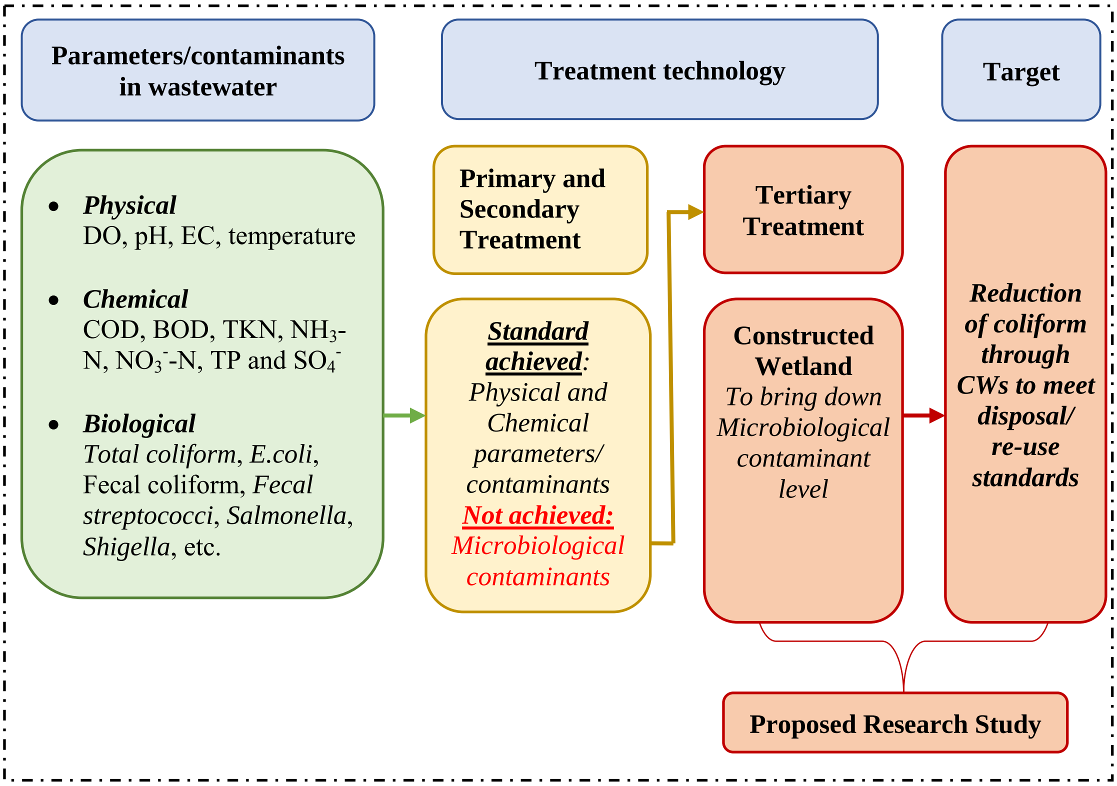

Fig. 1.2. Theoretical framework – Target of the proposed research problem

1.3 Need for the Proposed Research

Appropriate water reclamation and reuse technologies have been adopted as the most feasible alternatives to overcome the problems of freshwater availability by many cities around the world. Wastewater is found to contain variety of contaminants including microbial species which may be present at levels posing great risks for public health (Hagendorf et al., 2000; Belmont et al., 2004; Vacca et al., 2005). The presence of microbial contaminants has also been seen in disposal of inadequately treated wastewater or in wastewater discharges which are actually sourced from excretion of disease carrying humans and animals (Sleytr et al., 2007;Wen et al., 2009). So far, the treatment technologies employed in the conventional wastewater treatment plants are often based on the Activated Sludge Process (ASP). The performance efficiencies of these treatment plants are limited to organic matter and nutrient removal only, while 2-3 log reduction of coliform is incidental.

In India, estimates reveal that 38,354 million liters per day (MLD) sewage is generated in major cities of the country, while there exists a sewage treatment capacity of only 11,786 MLD. Though the wastewater treatment capacity in the country has increased by about 2.5 times since 1978-79, yet hardly 30% of the sewage generated is treated effectively, while the rest either sinks into the ground as a potential pollutant of ground water or is discharged into the natural ecosystems and causes large-scale pollution in downstream areas such as rivers and ground water (Control of Urban Pollution Series: CUPS/70/2009-10; Kaur et al., 2012; Trivedy and Nakate, 2001).

Considering all these grave issues, the government of India established 234 Sewage Treatment Plants (STPs) but their effluents are not usually suitable for household purposes and reuse of the wastewater is mostly restricted to agricultural and industrial purposes only (CPCB 2005). The direct exposure and release of effluent containing pathogens for irrigation of agriculture and horticulture crops and other reuse purposes may pose potential health risks and restricts their use for agriculture and domestic purposes. Thus, the associated health risks in their effluent is a matter of concern. It has now been directed by government authorities to limit the *Total coliform* numbers as suggested by WHO i.e. 1000MPN/100ml for irrigation and safe disposal into water bodies. Extensive efforts made by the government of India on a regular basis to evaluate the performance efficiencies of the treatment plants confirm that the effluent contains excess concentrations with 10^4 to 10^6 MPN/100ml of *Total coliform* (CPCB, 2008; Jamwal and Mittal, 2009). Such outfalls in terms of microbiological contaminants associated with treated effluents threatens the life forms of the receiving water bodies during discharge. Hence there is a need for as tertiary treatment of the treated effluents that do not meet the prescribed standards and pose potential health risks

(Carr et al., 2004; Kracman et al., 2001: Schaefer et al., 2004; Koivunen et al., 2003; Sonune and Ghate, 2004; Kaur et al., 2011).

Researchers are still struggling to develop an appropriate, economically viable and environmentally sound tertiary treatment processes to remove microbial contaminants from wastewater. There are many conventional treatment methods that are used to treat secondary treated effluents such as using chlorine gas, liquid chlorine, ozone, UV disinfection, etc. In India, majority of the plants equipped with disinfection systems are using gas chlorination. A contact time of 30 minutes is provided in the chlorination tank to disinfect the treated sewage with varying chlorine dosages. Chlorine gas disinfection, being cheaper is preferred over ozone and UV disinfection. However, it has certain disadvantages like formation of tri-halo-Methane (THMs), corrosion in chlorination tanks, and requirement of highly mechanized system to handle chlorine gas and a need for a de-chlorination unit, in case treated wastewater is discharged into rivers. All the conventional treatment systems (chlorination, UV disinfection and ozone) are machine intensive with limited life, high installation charges and high operation and maintenance costs which make them unsuitable and unaffordable and restrict the government in their establishment in developing countries (Kivaisi, 2001; Belmont, et al., 2004; Kadam et al., 2008; Boutilier et al., 2009; Ren, 2010).

Due to these limitations of the machine systems, natural systems for reduction of microbial species, such as wetlands systems are gaining importance. They improve the water quality through a combination of physical, chemical and biological processes (Kadlec and Knight, 1996) and are found to be low cost, effective, eco-friendly and thus a natural alternative to technical methods of wastewater treatment (Vymazal, 2005; Hofmann, 1996; Denny, 1997; Stottmeister et al. 2003; Brix et al. 2005; Seo et al. 2005). Therefore, well-known constructed wetlands are engineered wetlands that are built to emulate the functions of natural wetlands for human needs. They offer physical, chemical as well as biological mechanisms in a single treatment system. Constructed wetlands seems to have firmly established roots in terms of wastewater treatment due to their high pollutant capturing capacity, simplicity, low energy demand, process stability, low excess sludge production, effectiveness and potential for creating biodiversity (Vymazal et al., 1998; Korkusuz et al., 2004; Kadlec and

Wallace, 2009). Hence, constructed wetlands can be optimized for advance wastewater treatment for the purposes of urban reuse especially in areas where water demand is high.

Among the two types of constructed wetlands, horizontal and vertical flow constructed wetlands, the vertical sub-surface flow constructed wetlands have some major and important advantages over horizontal flow constructed wetlands - more and uniform root distribution and water-root contact, fewer problems of bad odor and lesser proliferation of insects (since they do not have free surface), sequential facilities of anaerobic and aerobic environment, higher oxygen transportation in turn leading to higher removal of organic matter and greater nitrification through the activity of ammonia oxidizing bacteria (Brix and Scierup, 1990; Haberl et al. 1995; Platzer and Mauch, 1997; Cooper 1999; www.wepa-db.net; Cooper 2005; Kayser & Kunst 2005).

Use of constructed wetlands for the secondary treated effluents increases the expectations for possibilities of better performance of the treatment systems. A study on STPs in Delhi (Jamwal and Mittal, 2009), carried out to evaluate the treatment efficiencies, suggests 119±32 to 450 mg/l BOD and 172±49 to 666±98 mg/l COD in raw sewage while 2 to 90±18 mg/l BOD and 21±9 to 146±20 mg/l COD in treated effluents. Thus the reduced organic load in treated effluents supports the biological elements of the wetland functions to work properly, which ultimately increases the life of the system by reducing the rate of clogging, which is a problem associated with constructed wetlands treating only pre-treated domestic/municipal or industrial wastewater. It is in agreement with the study by Hench et al. (2003) which clearly restricts the use of constructed wetlands as the sole treatment option for pre-treated wastewater.

Much effort in the recent years have been focused on removal of microbial contaminants from domestic or municipal wastewater through vertical flow constructed wetlands where the coliform concentration in influent was found to be around 8 log CFU/100ml (Hench et al. 2003; Vacca et al. 2005; Slyetr et al. 2007; Kadam et al. 2008). In such cases, the removal of microbial contaminants has been mainly observed in primarily treated wastewater and the resultant higher removal efficiency was directly correlated with their high microbial influent concentrations (Vymazal 2005; Ulrich et al. 2005).

So far, the removal of microbial contaminants with lower influent concentrations is of lesser concern.

1.4 Research Development

The research work in the present study focuses on the use of vertical up-flow (VUF) regime for removal of microbial contaminants from the treated effluent of a STP. Within the domain of the study three aspects were considered, (a) to identify the sustainability and suitability of vertical up-flow constructed wetland (VUFCW) for removal of pollutants from secondary treated effluent, (b) to select a suitable plant species among the conventionally used plant species and locally available plant species and (c) to achieve maximum removal of coliform from treated sewage effluent to meet the corresponding standards. This study is directed towards proposing an eco-compact technology that can be used for further treatment of treated effluent in order to reduce its associated health risks in similar prevailing climatic conditions where large land availability is a major constraint. Moreover, the components of the whole experimental set up were selected to be low cost with ease of availability according to their applicability on field scale. Also it was contemplated that the research would be useful and informative for consideration by government bodies and professionals while framing an integrated approach for tertiary treatment of secondary treated wastewater discharged from ASP based treatment plant. It was ascertained that the research would also add some knowledge to the reported literature for selection of suitable plant species in semi-arid climatic conditions, to bring down the coliform count within the prescribed standards (1000MPN/100ml by WHO) in secondary treated wastewater.

1.5 Aim and Objectives of the Study

The aim of this study was to reduce the health risks associated with the disposal of secondary treated effluent into water bodies. Within this aim, the objectives were identified as follows:

1. To assess the survival and growth of locally available plant species in the secondary treated wastewater for semi-arid climatic conditions.

2. To analyze the performance of laboratory scale experimental constructed wetlands with reference to removal of organics, nutrients and microbial contaminants from secondary treated wastewater.

3. To identify suitable plant species for contaminants removal from secondary treated effluent.

4. To assess the role of media on performance of laboratory scale constructed wetland units.

5. To identify best performing constructed wetland with the aim of achieving the standard limits for coliform.

1.6 Scope of the Study

The scope of the work was limited to:

1. Construction of the pilot scale experimental set-up, consisting of three constructed wetland filter/treatment units. Two of them were planted while one was kept without plantation and called "Control".

2. Characterization of secondary treated wastewater that comes out from Sewage treatment plant at south Jaipur based on activated sludge process.

3. Identification of locally available and suitable species for vegetation for establishment of CWs units to polish secondary treated wastewater.

4. Experimentation and subsequent comparison performance of these macrophyte with respect to the behavior (growth pattern and sustainability) when the effluent is applied.

5. Investigation of removal efficiencies of constructed wetlands for pollutants like pH, dissolved oxygen (DO), Chemical oxygen demand, Total-Kjeldahl-Nitrogen, Ammonia-N, nitrate-N, *Total coliform, Fecal streptococci and Salmonella typhi*.

6. Study of the impact of incorporation of soil layer into filter bed of constructed wetland regarding removal efficiency of microbial (*TC, FS and S.typhi*) species.

7. Statistical analysis (two tailed t-tests) in order to prove the differentiations about performance of different constructed wetlands

1.7 Organization of Thesis

The thesis is divided into five Chapters. Chapter 1 is the current introduction chapter presenting the background and need of the presented research. The chapter includes overall aim of the study and several objectives that were identified within the aim of the study. The chapter also give insights on the way in which the thesis has been organized.

Chapter 2 - Provides the basis on which the components were selected for the whole experimental setup. It provides the background information on the design of the experimental setup including overview of the factors affecting organizational activities in the context of the set ups of constructed wetland for the proposed research. The literature review and the conceptual theoretical framework are presented in this chapter. At the end of this chapter, summary of the identified research gap is discussed which is based on literature reviewed throughout the chapter.

Chapter 3 - Describes the materials and methods used to construct and run the experimental set up of vertical up-flow constructed wetland. The details of experimental units are elaborated by presenting best possible manner through relevant pictorial diagrams, figures and table of components. Description of techniques and protocols used in analysis of parameters is indicated by giving references from where they have been followed and briefing of steps followed is also mentioned in the chapter.

Chapter 4 - Presents the results obtained during the experimentation of all constructed wetland units during the study period. The chapter provides detailed analysis of result about the performance of the CW units including discussion with reported theories. Data analysis was performed through laboratory as well as statistical analysis tools. Finally the inferences about functional activities of treatment systems were made by correlating the microbial removal results with background analysis, to identify the most governing removal mechanisms for microbial contaminants.

Chapter 5 - Presents the results obtained from the characterization of biofilm of all constructed wetland units carried out at the end of the experimental study. The chapter accounts for the comparative analysis of richness distribution and diversity including the description of identified community composition among rhizosphere and different media layers.

Chapter 6 - Finally concludes and summarizes the study and presents them as appropriate final results drawn from analysis of data. It gives suggestions on the basis of results and recommendations for possible further research to carry forward the study.

Finally the thesis concludes with the list of references and list of publications and biography of the researcher.

Chapter 2
Literature Review

2.1 Introduction

The chapter includes a detailed literature that have been reviewed to address the proposed research problem. The review primarily gives insights about the constructed wetland technology as well as its classification. Further details about the components related to its configuration are also well studied and stated in the chapter. A thorough review about vegetation is presented including details of filter media. After explaining the configurable details, the microbiology associated within constructed wetland system is discussed. The removal mechanism of all contaminants is also explained in the later part of the section of this literature review chapter. In the last section of the chapter, summary of the literature and knowledge gap are documented based on the review.

To start with introducing research problem in brief, it has been found that growing awareness of environmental-pollution-issues has urged government to enforce water and wastewater management practices because consistent sewage discharge creates serious environmental stress on water resources. In this purview, the authorities have tried out different wastewater treatment plans over the worldwide. The use of wetland systems as a polishing unit for conventional treatment plants is not new infect long life span of CWs could be expected while being used as a post treatment unit. It has been seen that the application of untreated wastewater such as raw sewage and industrial waste should not be used so that the biological components of wetland system can play efficiently (US EPA, 2000; Thaddeus and Frances, 2007). In general, wetland receives, hold and recycle nutrients and support vegetation at microscopic as well as macroscopic level (Crites and Techobanoglous 1998; Hammer 1989).

2.2 Constructed Wetlands

Constructed wetlands are relatively less expensive to construct and operate with ease of maintenance and with provision of effective and reliable wastewater treatment. Wetlands are found to be relatively tolerant to fluctuating hydrologic and contaminant loading rates. At the beginning of the 20^{th} century the CWs were thought to be permanent fixture in the areas where financial crises are of concern.

The concept behind the use of wetlands was firstly introduced in 1950's by Seidel who named it "hydro-botanical system" (Vymazal 2011). This system was used for the removal of pollutants from wastewater. It was further improved by following the hybrid system of vertical and horizontal filtration beds (Seidel, 1992). Afterwards better understanding about wetland engineering, design, ecology and biology was given by Vymazal, 1996, Kadlec and Knight, 1996 and Hans Brix through extensive studies and are still continuing. The wetlands were considered as transition between terrestrial aquatic ecosystem as well as the serial stages in succession from land to water or vice versa (Gopal and Sharma, 1982). The ecosystem of wetland systems has the specific characteristics which makes them suitable for wastewater purification: (1) the semi-aquatic system with partial oxic and partial anoxic environment. (2) Provides basis to support the tall and highly productive plantation for taking up the nutrients. (3) And enhance the growth of the microbial film supported on media (Verhoeven, Meuleman, 1999). Besides wastewater treatment capacity of these systems provides ancillary benefits including: green space, food and wildlife habitats, water quality improvement, flood protection, shoreline erosion control and recreational & educational area, aesthetic appearance, pleasant vegetation as well as able to produce plant biomass, generally which are not present in conventional treatment plants (Frooqi et al., 2008; Knight, 1997; Jillson et al., 2001; Shutes 2001and Wetzet, 2001).

These specifications have been portrayed into an artificial model to maintain the optimum environmental condition and which is termed as a "Constructed Wetland". The constructed wetlands are also known as reed beds which describe sub-surface flow constructed wetland. In 1970s and 1980s, these systems were introduced as a result of concern for treatment of municipal or domestic wastewater. Since 1990s, it has been used for almost all types of wastewater such as landfill leachate, runoff, food processing, industrial, agriculture farms, main drainage or sludge dewatering (Farooqi et al., 2008). Kadlec and Knight (1996) define that the constructed wetland systems are designed to utilize natural process for wastewater quality improvement. The constructed wetland systems are engineered systems to facilitate the purification process by removing contaminants in wastewater via a combination of physical (filtration, sedimentation) chemical (precipitation, adsorption) and biological (microbial activity, vegetation accumulation, plant uptake) processes for different type of wastewater. They are design to take advantage of many of the same processes that occur in natural wetland, but do so within a more controlled environment (Vymazal, 2005). Constructed wetlands are found to be promising for removal of TS, TDS, TSS, COD, BOD, TKN and total phosphorus as well as microbial contaminants (Gersberg et al., 1994; Green, 1994; Kadlec, 1989; Knight, 1987).

2.2.1 Classification of constructed wetlands

The different types of Constructed Wetlands mainly depend upon the flow regime i.e. free water surface flow CW and sub-surface flow CW. These categories can be further classified on both the type of macrophytic growth and on the water flow direction (Figure 2.1).

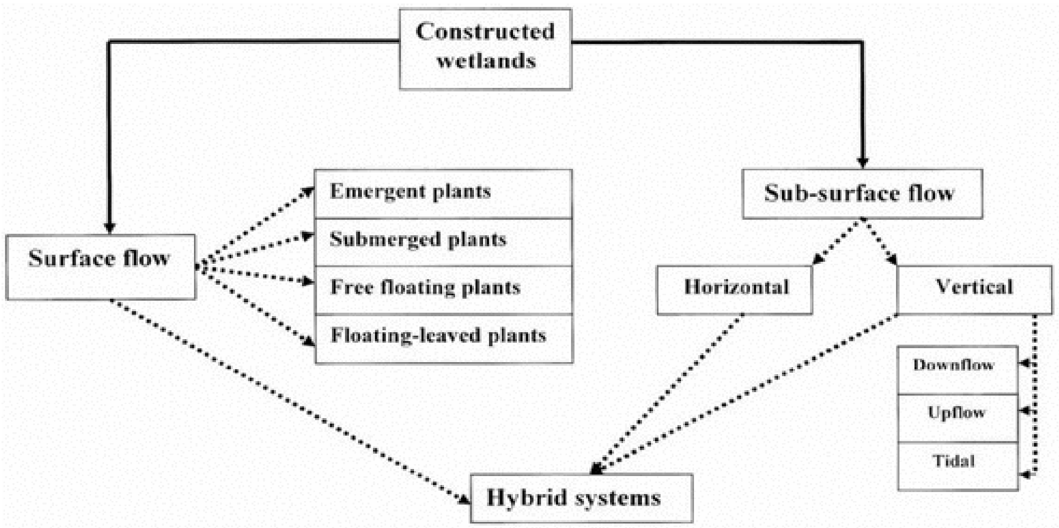

Fig. 2.1. Classification of constructed wetlands (Vymazal and Kröpfelová, 2008)

2.2.1.1 Free water surface flow constructed wetlands with emergent macrophyte

Surface flow constructed wetland is a shallow sealed or sequence of basins which contains rooting soil with a permanent water depth (Vymazal, 2010). In FWS systems, water always remains above the ground and plants are rooted in the sediment layer at the base of water column. Reeds et al. (1988) ensures the plug-flow condition due to shallow water depth and low flow velocity. A typical free water surface constructed wetland has emergent macrophyte containing 20-30 cm of rooting soil in which flow is directed into a cell along a line comprising the inlet, upstream embankment and intended to proceed to all parts of the wetland to one or more outlet structure/s (Vymazal, 2008). The presence of plant stalks and litter regulate the water flow especially in long-narrow channels. The design of free water surface flow allows water to flow with landscape is similar to natural wetland facilitating a permanent contact of wastewater with biologically reactive surface of the system (Kadlec and Knight, 1996).

Free water surface constructed wetland with emergent macrophyte functions as land-intensive biological treatment system. The large requirement of dimensions often resembles swamps and marshes with diverse ecology (Gray, 2004). Inflow water containing particulate and dissolved pollutants spreads through a large area of shallow water and emergent vegetation (Kadlec and Knight, 1996). In these types of wetlands, algal and microbial uptake may be high but retention is a short-term process and nutrients are washed out from detritus back to the water. The most commonly used emergent are *Phragmites australis, Scirpus lacustris, Typha latifolia, Scirpus, Sagittaria latifolia* etc.

2.2.1.2 System with free floating and submerged plants

Constructed wetlands with free floating plant consist of one or more shallow ponds in which plants float on the surface. The shallower depth and the presence of aquatic macrophyte in place of algae are the major difference between constructed wetlands with free floating macrophyte and stabilization ponds (Kadlec et al., 2000). Due to the economic concerns, these types of wetlands are not found appropriate for wastewater treatment. Stewart et al. (1987) reveals that the use of water hyacinth and water lettuce is strongly temperature-dependent and the use is restricted to subtropical and tropical regions.

In free water surface flow constructed wetlands with submerged plants, the photosynthetic part of the plants always remains entirely submerged in the water. According to the study by Brix (1993) these plants cannot be used in wastewater treatment because they grow well only in oxygenated water. Sahai and Sinha (1976) reported that long exposure to low oxygen concentrations may reduce growth of submerged macrophyte. Such statements make their use of less concern as a treatment options for wastewater.

2.2.1.3 Sub-surface flow constructed wetlands

The subsurface flow constructed wetlands allow the water passage within the substrate: below the surface level, which could move in horizontal or vertical direction throughout the system. In SSF-CW there is no direct contact between the water column and atmosphere. These systems are engineered to stimulate and optimize natural wetlands. In this type of CWs opportunities for vermin to breed is very less or negligible which makes the system safer from a public health perspective.

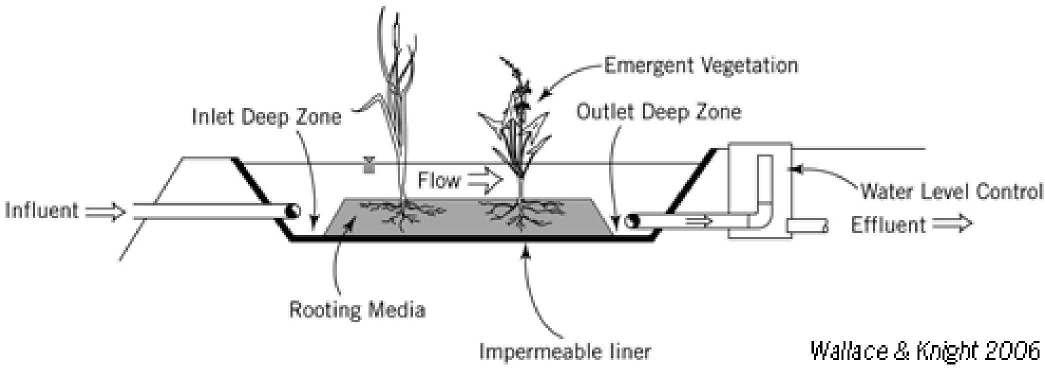

Fig. 2.2. Free water surface flow constructed wetland (Wallace & Knight, 2006)

2.2.1.4 Sub-surface horizontal flow constructed wetland

In Sub-surface Horizontal flow constructed wetlands water is allowed to pass throughout media or substrate in horizontal direction. Water is fed at the inlet and flows horizontally below the surface with consistent contact of root-zone and with the bio-film associated onto the substrate (Vymazal, 2008). During the movement of water it comes into contact with three different zones i.e. aerobic, anoxic and anaerobic zone. The aerobic-zone existence is defined by the leakage of oxygen into the substrate by rooting parts (Cooper et al., 1996). Following the aerobic zone, anoxic and anaerobic zones occur due to insufficient oxygen transport (Vymazal and Kropfelova, 2006). So far, these systems are found to be good for denitrification but not for nitrification. The large land area requirement for establishment of horizontal flow constructed wetland limits its approach.

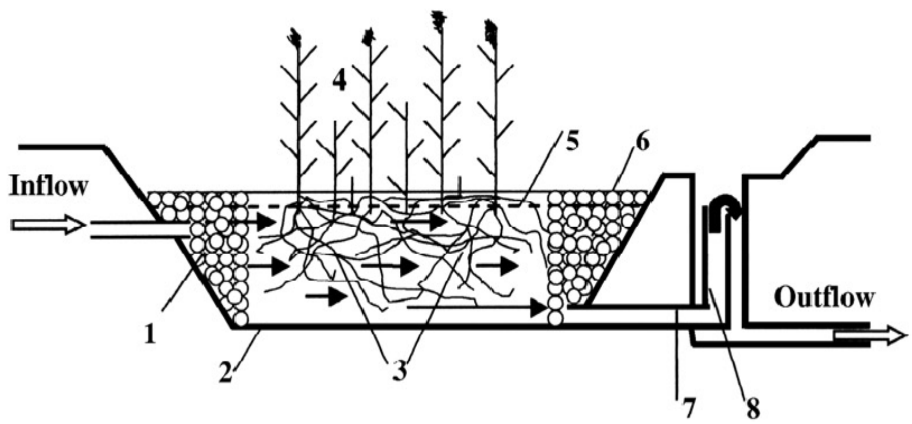

Fig. 2.3. Horizontal sub-surface flow constructed wetland (Vymazal, 2001)

2.2.1.5 Sub-surface vertical flow constructed wetland

The first design of vertical flow constructed wetland was given by the Seidel (1965) while using it in combination with horizontal flow for wastewater treatment at Max Planck Institute in Germany (Vymazal, 2009). The wastewater feeding in the vertical flow constructed wetland is intermittent in which wastewater gradually percolates down/up through bed. This type of feeding provides good oxygen transfer and the ability to nitrify (Cooper et al., 1996). In the past decades, vertical flow constructed wetlands were used as a first stage of hybrid systems (Burka and Lawrence, 1990; Lienard et al., 1990) which is now being used as single or multiple vertical bed system. These systems are called 2nd generation vertical flow constructed wetlands or compact vertical flow beds (Weedon, 2003; Brix et al., 2002; Arias and Brix, 2005). For these systems, high oxygen content and better substrate aeration has been reported. Vertical flow beds provide minimal denitrification so that ammonia–N is usually only converted to nitrate-N as there is good nitrification facilitated by the presence of sufficient oxygen. Therefore, overall low removal of nitrogen has been seen compared to HF constructed wetland. The vertical flow wetland can be sub divided into two categories based on direction of water flow i.e. vertical down-flow and vertical up-flow constructed wetland (Figure 2.4 and 2.5, respectively).

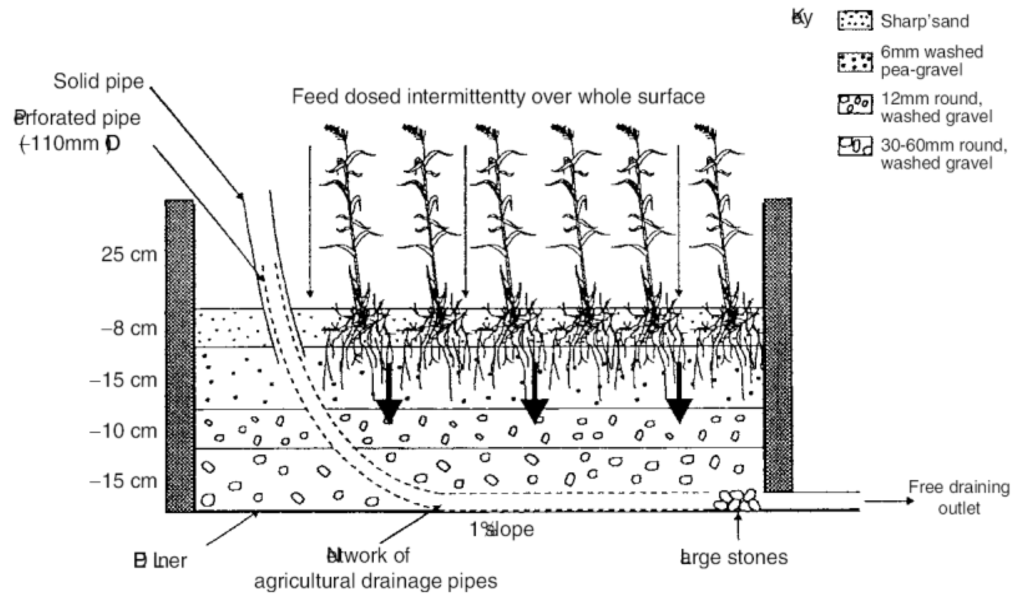

Fig. 2.4. Typical arrangement of a vertical down-flow constructed wetland (Cooper et al., 1996)

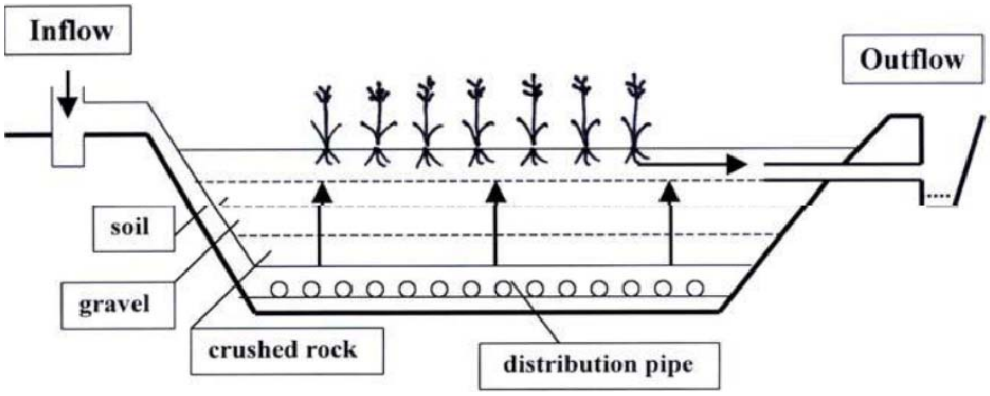

Fig. 2.5. Schematic representation of a constructed wetland with vertical up-flow (Vymazal, 2001)

Advantages of vertical flow system are provision of good oxygen transfer, better removal of organics, suspended solids, and ammonia. These types of wetlands require less land as compared to horizontal flow constructed wetlands. The designing advantages of the vertical flow CW over the other designs are that they have more equal root distribution and water-root contact and limited problems related to odor and proliferation of insects as there is no free water surface for their catchment (Haberl et al., 1995; Cooper, 1999). On the other hand, the consequences associated with this type of flow are requirement of more maintenance and operation efforts because of the use of pumps, timers and other electric and mechanical devices. According to Cooper (2005), the most important factors to achieve in the design of a vertical flow are:

1. To produce a bed matrix that allows the passage of the wastewater through the bed before the next dose arrives whilst holding the liquid back long enough to allow the contact with the bacteria growing on the media and achieve the required treatment.
2. To provide sufficient surface area to allow the oxygen transfer to take place and sufficient bacteria to grow.

2.2.1.6 Hybrid system

Hybrid systems are developed while using combination of horizontal flow and vertical flow constructed wetland for wastewater treatment at Max Planck Institute in Germany. It is aimed to combine the advantage of both the systems and complement their processes to one another. The overall removal of nitrogen can be achieved by using hybrid system in which nitrification can be better achieved by vertical flow CW rather than HFCW while good denitrification occurs in HFCW than VFCW.

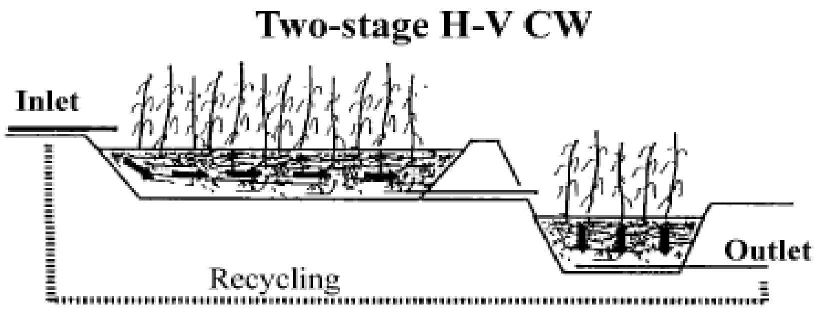

Fig. 2.6. Hybrid constructed wetlands

2.2.2 Components of constructed wetlands

2.2.2.1 Vegetation

The use of constructed wetland actually came from the basis of benefits/facilities provided by vegetation. In 1982, it was thought by W.R. Duffer that aquatic macrophyte-based systems (AMATS) can reduce cost of secondary and advanced domestic wastewater treatment than conventional treatment system. Early experiments of using constructed wetlands for treatment of wastewater were based on vegetation i.e. “Root zone Method” and “Hydro-botanical method”. Such system was found to be a viable alternative to the conventional technologies available for wastewater treatment (Brix, 1987; Vymazal, 2005). Vymazal et al. (1998) included the classification or type of the wetland plants (Wetzel, 1983): emergent aquatic macrophyte, floating leaved aquatic macrophyte and submerged aquatic macrophyte and stated that the plants as indispensable components of these systems. Kadlec et al. (2000) found vegetation as an essential component for wetland systems, even selection of suitable plant for vegetation in wetlands plays critical role in optimizing the system for highest removal of contaminants (Reddy et al., 1989).

Being a major part of the system, the prime importance of vegetation has been observed for removal of nutrients especially through extensive research from past decades and still continues (Brix 1997; Koottatep and Polprasert 1997; Kivaisi 2001; Clarke and Baldwin 2002; Matheson et al. 2002; Kyambadde et al., 2004; Mbuligwe 2005; Greenway, 2007; Zhang et al., 2007; Vymazal and Kröpfelová, 2009; Liang et al., 2011; Chang et al., 2012). Still, the significance of vegetation cannot be neglected for removal of other physiochemical and microbiological contaminants from wastewater.

Role of vegetation

Phytoremediation is the key mechanism facilitated by plants which is of major importance for their applicability in treatment of wastewater (Bose, 2008). In general, plants can be divided into two well defined parts i.e. above ground: shooting part and below ground: root zone. In general, shooting parts of the plants provide shade and protection against cooling whilst the root part offers a habitat for organisms and makes the media permeable. At the rooting zone of Plants, diffusion of atmospheric oxygen into their root zone within the wetland system occurs via arenchyma plant tissue and create aerobic zone, anoxic and anaerobic zone around the zone (Reddy et al., 1989; Reddy and DeBusk 1987; Brix 1997). Therefore, it has been found to be most active-reaction zone due to interaction of microorganisms, plants, media and pollutants in this particular zone (Ulrich et al., 2005; Sttomeister et al., 2003). Moreover, certain advantages facilitated by plant roots are: large surface area for microbial colonization, substrate for microbial growth, supply of reduced carbon (Kadlec et al., 2000) and oxygen to the rhizosphere and shading off of algae. They also help to decrease current velocity, stabilize the surface of the bed, and insulate the surface against frost winter (Brisson, and Chazarenc et al., 2009). Additionally, plants also alter the hydrologic budget via evapotranspiration, provide wildlife habitat, and act as a natural filter for suspended solids. They take up nutrients as well, though the extent of this may be insignificant, and certain pollutants like phosphorus are re-released with the decay of plant litter.

The role of plants for pollutant removal has been extensively studied for wastewater treatment and it is found that the pollutants are assimilated directly into the plant tissues (Thallen et al, 2005), act as a catalyst for purification reactions, and increase the diversity in the rhizosphere which promote a variety of chemical and biological reaction and enables the removal process (Kadlec and Knight., 1996). Above mentioned review suggests that the removal of nutrient accomplished by plant uptake, assimilation, nitrification-denitrification activities at rooting zone via microbial processes which are ultimately supported by plants parts. Plant role mostly affects the nutrient removal and also helps indirectly for organic matter removal. They are also helpful for removal of microbial contaminants from wastewater.

Vegetation and microbiological contaminants removal

Many previous studies (Gersberg et al., 1989b ; Rivera et al., 1995; Soto et al., 1999; O. Decamp et al., 1999) supports that the significance of vegetation in removal of microbial contaminants. Many researches correlated the bacterial die-off with dissolved oxygen content (Fernandez et al., 1992; Pearson et al., 1987). Some other studies suggested that the reduced dissolved oxygen concentration in the root zone which in turn lowers the microbial activity causes the lower reduction of microbes (Armstrong et al., 1990; Rivera et al., 1995). While studies of 20th century such as Hench et al. (2003), Vymazal, (2005), Wand, (2007) and Chang et al. (2010), also observed better removal in planted wetland. Such studies favors the usage of plants for the removal of microbial contaminants via the oxidation and exposure with released bactericidal substances including antimicrobial activities of root exudates (release of antibiotics may kill or control the bacteria in the wastewater) (Kickuth and Kaitzis 1975; Mendi et al., 1993; Axelrood et al., 1996; Stottemeister et al., 2003; Bai et al., 2004; Li et al., 2004; Molleda et al., 2008). Other roles of plants for removal of microbial contaminants were related with providing increased surface area for adherence and for sedimentation, filtration and habitation for protozoan species etc. (Gersberg et al., 1987; Gerba et al., 1999; Soto et al., 1999). A study by Gracia et al. (2008) observed better bacterial removal efficiencies in planted systems than in control systems and reasoned that macrophyte may be contributing to bacterial die-off by means of indirect effects such as conductivity modification, gas transport and enhancement of biofilm development, adsorption, aggregation and filtration which is in concordance with other reported theories (Kadlec and Knight, 1996; Brix, 1997; Ottova et al., 1997).

While some of the contradictory observations in this regard have been given by Vecca et al. (2005), Keffala and Ghrabi, (2005); Sleytr et al. (2007) and Torrens et al. (2009) where vegetation is of minor significance. These contradictory observations could be correlated with such statements: shading of vegetation may interfere in exposure of UV light (Quiñónez-Díaz et al., 2001) and/or enteric bacteria may become part of rhizosphere's community of (*Phragmites* spp.) plant (Vacca et al., 2005).

The most commonly/frequently used plant species for wetland establishment worldwide for subsurface flow constructed wetland is *Phragmites* spp. (Kadlec et al., 2000). Through the literature review as above it has already been revealed that mostly the two emergent species e.g. *Phragmites* spp. and *Typha* spp. have been seen in temperate climate for treatment wetlands (Reddy et al., 1989). Their use in treatment wetlands is still continuing (Hench et al., 2003; Vacca et al., 2005; Lee and Scholz, 2007; Molleda et al., 2008; Torrens et al., 2009).

In recent past, extensive use of *Canna indica* as a wetland vegetation species has been seen in China along with some other countries (Calheiros et al., 2007; Naz et al., 2009; Cui et al., 2010; Liang et al., 2011; Chang et al., 2012). *Canna* spp. is found to be a suitable plant species for wetland construction in developing countries by representing an economic alternative (Zurita et al., 2006). A review by Brisson and Chazarenc et al. (2009) gives plant species-comparison, and reported that *Canna* was found to be applicable for wetlands. A study by Dennis Konnerup et al. (2009) suggested its suitability in domestic wastewater. The specie has been found with healthy and vigorous growth, highly effective to remove nutrient resulting from high potential of root-zone aeration capability with rapid growth rate, large biomass and beautiful flowers (Zhang et al., 2007; Liang et al., 2011). Also, *Canna indica* is known as phytoremediation plant (Bose et al., 2008), having a flourishing root system with higher root growth, higher root number, larger root biomass and significantly larger root surface area than other plant species. This plant has high tolerance to the pollutants and clogging, and has long root life span (Wenyin et al., 2007; Li et al., 2008).

A study that could be in favor of statement given by Tanner, (1996), suggested that aboveground biomass was more strongly correlated to total nitrogen removal than below ground biomass. While other parameters which are usually considered are root surface area and leaf surface area. Root surface area is especially related to microbial colonization and leaf surface area is related to better reflection for oxygen transport to roots than plant biomass (Tanaka et al., 2007). Faster growing plants with higher number of roots were considered as favorable for nitrifying bacteria which lead to increased nitrification (Kyambadde et al., 2004; Liang et al., 2011). Such facts suggest vegetation with *Canna spp.* in constructed wetlands. Among all above stated theories, where *Canna* plantation was used, only few of the studies observed the removal of microbial contaminants (Zurita et al., 2006; Chang et al., 2010).

Contradictory statements for vegetation in constructed wetlands

Gercia et al. (1997) report greater removal in planted wetlands than un-planted. Sleytr et al. (2007) did not find any significant difference between planted and un-planted for removal of total and heterotrophic plate count but for *Enterococci coli* it was significant. Vacca et al. (2005) favors the presence of plants for removal processes. Many studies such as Decamp et al. (1999) described the role of vegetation in increased removal of contaminants by facilitating the improved aeration within the root zone. Moreover, some studies showed the release of bactericidal substances from root exudates for removal of microbial contaminants (Ottova et al., 1997). Likewise, from vegetated systems, removal of microbial contaminants is also favored by observing higher predation in comparison to non-vegetated system (Wand et al., 2007). In this regard, high removal efficiency through vegetated systems has been proved in other reported studies as well (Rivera et al., 1995; Wand et al., 2007).

In contrast, study by Vacca et al. (2005) found lower bacterial contaminants removal from *Phragmites australis* planted system than the unplanted systems. Torrens et al. (2009) didn't find significant difference between planted and un-planted systems. Tracer tests for these studies confirmed that planted and un-planted systems show same flow-patterns with similar mean HRT. Author revealed that in intermittent sand filters oxygen transfer is mainly due to intermittent feeding and diffusion of air and by convection.

2.2.2.2 Filter media

Being a major component of the wetland systems filter media provides support to the whole constructed wetland system by facilitating porosity within the system, allowing water to flow or move within the system, supporting the vegetation to stand well as well as roots to penetrate throughout the medium and most importantly provide surface area for bio-film to develop (Stottmeister et al., 2003). Moreover, a hand book on constructed wetland (Volume 1) stated that substrate sediments and litters facilitate space for living organisms, sites for material exchange and microbial attachment, source of carbon and energy, space for diffusion of oxygen and many chemical and biological reactions to occur. As far as different media types are concerned, soil, different sized gravels, sand peat and compost are most commonly used media for subsurface wetlands (IWA 2000; Weber and Legge 2008). A brief review on use of different filter media correlated with the performance efficiency is listed in Table 2.1 and enables to suggest good agreement for proposed research with above mention statements.

Table 2.1

Summary of literature presenting use of different media including their respective sizes in constructed wetland with different types of wastewater and resulting corresponding removal for contaminants

S. No.	Country	Type of wastewater	Media type	Media arrangement (from top to bottom)	Removal efficiency	Plant species	References
1.	Thailand	Synthetic wastewater	Sand and gravel	20cm Sand(1-2mm) 15cm Gr.(4-12mm) 15cm Gr.(30-60mm)	-	*Typha spp.*	S. Kantawanichkul et al., 2009
2.	China	Household domestic wastewater	Sand and gravel	90cm sand (0.4-1mm) 15cm pea Gr. (5-12mm) 15cm pea Gr.(10-30mm)	BOD-96% TSS- 97% NH_4-N- 88.4% TP-87.8%	*Salix babylonica*	S. Wu et al., 2011
3.	Italy	Municipal wastewater	Sand, gravel	16cm Sand (0-0.16 mm) 22cm Gr. (4-8mm) 22cm Gr. (8-12mm) 90cm Gr. (50-80mm)	COD, BOD N,& K - >86% Na & Mg < 47%	*Typha* and *Phragmites spp.*	Morari, F and Giardini, L. (2009)
4.	Greece	Olive mill wastewater	Sand and gravel	I-17cm Gr.(6mm) 7cm Gr. (24mm) 7cm cobbles (60mm) II- 6cm sand (0.5mm) 9cm Gr. (6mm) 6cm Gr. (24mm) 9cm cobbles (60mm) III/IV- 11cm sand (0.5mm) 3.5cm Gr. (6mm) 9cm Gr. (24mm) 5cm cobbles (60mm) (all are connected)	(overall effect only) COD-70% phenole- 70% TKN- 75% NH_{3-} uptp 74% orthophasphate -87%	*Phragmites spp.*	E.Herouvim et al., 2011

S. No.	Country	Type of wastewater	Media type	Media arrangement (from top to bottom)	Removal efficiency	Plant species	References
5.	Greece	Synthetic Domestic sewage	Sand, gravel	3cm Gr. (4-8mm) **42cm Sand (0-4mm)** 17cm Gr. (8-16mm)	COD-96% PO_4^{-}-52% TN->60% to nitrate	*Phragmites spp.*	C.A Prochaska, A. I. Zouboulis (2009)
6.	Pakistan	Wastewater from oil refinery	Gravel and Compost	I-15cm gravel 15cm sand II- 15 cm compost 15 cm sand	Gr. Based: BOD-35-69% COD-33-61% TS-39-56% Compost based: BOD-35-83% COD-45-78% TS-51-73%	*Phragmites karka*	M.M. Aslam et al., 2007
7.	Turkey	Olive mill wastewater	Sand, zeolite and gravel	8cm sand 10cm Zeolite 17cm Gr.	COD- upto 73% NH_4-N- upto 49% PO_4-P- upto 95%	*Typha latifolia* and *Cyperus alternatifolius*	A.Yalcuk et al.,2010
9.	Thailand	Domestic wastewater	Para-wood charcoal	Three different units of: charcloal (1mm,2mm and 3mm) surface area of each: 0.45m^2	BOD-95.5% COD-75% SS-94% TN- 92% TP-95%	*Typha spp.*	Thailand Sirianuntapiboon et al., 2007
10.	China	Lake water	Sand, gravel	55cm sand (0-4mm) 20cm Gr. (8-16mm)	TDS-31% COD-56% TN- 64% TP- 47%	*Acorus calamus* and *Canna indica*	ZHOU Qiaohong et al.,2009

Some recent studies for example Khalil (2009) used half burnt brick as a cheaper filter media and observed no adverse effects in terms of plant growth. Babatunde et al. (2011) used dewatered alum sludge cake as main substrate layer along with gravel layers and observed high phosphorus removal with high organic removal. Another study by Stefanakis and Tsihrintzis (2012) studied different types and sized media with different compositions. They used sand, gravels, river deposits, carbonate, zeolite and bauxite and their different combinations and did not find any significant improvement in pollutant removal. An Interesting feature that they observed was significant improvement of the system performance with using extended layer of sand above to the gravels.

So far it has been seen that the selection of media is depended upon pollutant that is being targeted. Organic matter, nutrients removal and microbial contaminant, are all affected by the use of filter media via filtration, sedimentation, adsorption, precipitation and accumulation processes. It can be concluded that the use of sand and gravels is conventional and is still majorly used filter media which can easily be available and economic to use in developing countries.

When media selection is seen in terms of microbial removal, it could be seen that filter media affects direct as well as indirect manner. Direct methods include sedimentation, adhesion & adsorption and mechanical filtration (Gersberg et al., 1989; Jillson et al., 2001; Scott et al., 2003; Karim et al., 2004; Keffala and Ghrabi, 2005; Weber and Legge 2008).

Some of the studies where the filter media is correlated with microbial removal are as follows: Rivera et al. (1995) found higher bacterial removal in sand bed than gravel bed. Pundsack et al. (2001) found peat media is more effective for removal of *Salmonella spp.* than sand media. Study by Koivunen et al. (2003) observed higher microbial removal in rapid sand contact filtration than chemical and biological contact filter. Arias et al. (2003) stated principal mechanism associated with filtration. Study by Gracia et al. (2003) studied the size effect of filter media using fine and coarse material for removal of *Somatic coliphage* and Fecal coliform and found higher attachment of pathogens in finer material. Likewise, a study by Sleytr et al. (2007), observed lower bacterial removal with bigger grain size. A unique combination or mixture was prepared and used as filter media by Kadam et al. (2008) to retain pathogens via filtration as well as adhesion. It was also suggested by the study that the media used in the study, helps to create unfavorable environment to sustain the pathogens within the system which in turn helped in removal of pathogens from domestic wastewater.

On the basis on all above literature review, it is clearly concluded that filter media plays an important role in removal of all type of contaminants from wastewater through constructed wetland regardless of type of wastewater, plantation and type of flow. It is to be noticed that each component has its own way to contribute for purification processes and these are not restricted to their individual contribution. They all are interrelated and perform in interactive manner to function at their best level.

With including above components of the constructed wetland system, the hydraulic retention time is also of major concern when the performance efficiency comes into consideration. Among all affecting components of the system, the retention time must be taken into consideration. Although its related studies that especially focus on microbial contaminants removal are only mentioned here, however, the role of hydraulic retention time is always observed as of significant effectiveness for the treatment of pollutants. Some of the reviewed studies are:

Bavor et al. (1989) suggested a 2 Day HRT at 20 degree Celsius to achieve a TC reduction to single log unit in unplanted CW while 3 day HRT *Typha* planted CW. Williams et al. (1995), found 2 log units reduction by using HST more than 1 day.

Gersberg et al. (1989) reported that the CW with *Scirpus* which receive secondary treated effluent reduces the Total coliform (TC) by 1.52 log units using 1/5 day HRT while it was found in the same study that 5.4 day HRT reduces 2.07 log units reduction in Mandi et al. (1993) reported that in sub-surface flow constructed wetlands, removal of Fecal coliform (FC) is achieved by 7 day HRT as opposed to 50 days required in macrophyte lagoons. Torrens et al. (2009) found the HRT plays an important role as at higher HRT; adsorption of microorganism would be enhanced which is one of the major mechanisms. He suggested that it's better to establish a correlation between minimum retention time with removal of FC than using mean retention time which would better represent the fraction of the dose applied that spends the least time in vertical filters. Role of HRT remains contradictory for last many years as it depends on influent concentration and its variation. Ottova et al. (1997), Tanner et al. (1996) and Mashauri et al. (2000) observed the positive relation between HRT and removal of FC and *E.coli* respectively, although the removal was not satisfactory as the best removal was of only 1 log unit in case of Mashauri et al. (2000) and Ottova et al. (1997) found lower removal of FC at lower HRT where wetland was based on only one type of vegetation and with fine grains of sand along gravels. Tanner found increased inactivation of FC at increased HRT. In this sequence Green et al. (1997) found the contradictory results where it was operated the pilot plants at five different HRT in two different experiments. In this study, it has been seen that at 2day HRT the removal of *E.coli* was lower than with 1 day HRT and in the second experiment the removal was lower with 5 day HRT than with 2 day HRT. This contradiction was reasoned by extraordinary variable concentration of bacteria in influent. According to Garcia et al. (2003) 3 day HRT works as a saturation point for microbial inactivation where saturation point is defined as point above which no increase in inactivation or no decrease in microbial population occurs. So far it is assumed that the first order assumption is invalid here. Thus it has been clear that the hydraulic retention time plays important role for removal of microbial species from constructed wetland and need to be appropriate.

2.2.3 Microbiology associated with constructed wetlands

To understand about the controllable factors which turns critical functional groups on and off, to be able to fully optimize the performance efficiency of the system and perhaps to be able to achieve the best suitable CW from domestic wastewater, there is need to understand the microbiology of the constructed wetland system. The microbiological understanding of constructed wetlands is of great importance as it directly related to the treatment performance of any system (Faulwetter et al., 2009). The transformation and mineralization of nutrients and organic pollutants are mainly driven by microbial species along with the plants within the constructed wetland (Aelion and Bradley 1991; Weisner et al., 1994; Stottmeister et al., 2003; Larsen E. and Greenway, 2004; Vacca et al., 2005; Faulwetter et al., 2009). The microflora comprises the biofilm which develops on plant root surface and media soil/particle (Watnick and Kolter, 2000). The growth of the microflora is of prime importance which is greatly affected by large amount of organic matter, nutrient and oxygen (Bapista et al., 2008; Qiaohong et al., 2009; Zhao et al., 2010).

Hence, it is required to provide appropriate substrate that could support the large amount of bacterial growth to construct efficient wetland (Qiaohong et al., 2009) because only well-developed biofilm could perform a variety of microbial activities and make efficient system (Greenway, 2007).

The microbial activities are encouraged with presence of plants where the rhizosphere produces more ecologically stable system and support larger microbial communities (Collins et al., 2004; Cristina and Calheiros et al., 2009; Zhao et al., 2010; Weber et al., 2008). Although the microbial communities may differ with different plant species at their rhizosphere as observed by Sleytr et al., 2009. In contrary, many studies did not find any significant impact of vegetation with change in bacterial population (Osem et al., 2006; Ahn et al., 2007; Bpista et al., 2008; Iasur-Kruh et al., 2010).

So far, microbial communities within the constructed wetland are greatly affected by environmental conditions, location and physic-chemical properties (Bapista, 2008; Sleytr et al., 2009; Iasur-Kruh et al., 2010). Except the vegetation and environmental conditions, factors affecting the bacterial population include depth and types of substrate provided within the wetland system. Tiez et al. (2007) stated that the >80% microbial productivity takes place within the first 10cm layer and Iasur-Kruh et al. (2010) found effect of depth on microbial community while Sleytr et al. (2009) did not find any significant difference in microbial community with filter depth. Some latest studies such as Cristina and Calheiros et al. (2009) and Ming et al. (2010) observed different microbial communities with different media.

In spite of factors affecting the microbial community diversity in the wetlands, only few studies concentrate on their identification. A study by Krasnits et al. (2009) studied the special distribution of major microbial group in HFCW, treating partially treated WW, and found the dominance of Eubacteria by presence of 85% followed by 50% presence of archaea and 40% of sulphate-reducing bacteria with almost negligible presence of nitrifying bacteria.

Study by Qiaohong et al. (2009) studied IVCW and found G-positive and G-negative bacterial population with presence of fungal biomass. They found aerobic, anaerobic and aerobic layer in sequence with dominance of *nitrosomonas* spp. in surface layer.

Study by L. Iasur-Kruh et al. (2010) found dominance of *acidobacteria, bacteroidetes, alpha, beta* and *gamma* subdivision of *proteobacteria* when the applied WW was treated from ASP (Activated sludge Process) based treatment plant.

Ming et al. (2010) found abundance of *aerobic prokaryotes*, in all eight filter-beds that were applied by mixture of lake and sewage contaminated water in septic tank. Qiaohong et al. (2009) found *nitrosomonas* like sequences and ammonia-oxidizing bacteria where the source of water was lake. Cristina and Calheiros et al. (2009) found bacterial community from genera of *Bacillus, Paracoccus, Pseudomonas* and *halmonas* where wetland was applied by tannery.

It can be concluded that microbially-mediated processes in constructed wetlands are mainly affected by hydraulic conditions, type of wastewater, type of substrate and nutrient availability, type of plant species, and many other environmental conditions. The same was also seen by Truu et al. (2009) during the review on different constructed wetlands for their microbial community structure and composition, it has also been found that the heterotrophic growth is higher in subsurface flow (vertical) than surface flow and the dominating species within microbial community of phylum *Proteobacteria*, followed by members of phylums *Cytophaga–Flavobacterium, Actinobacteria* and *Firmicutes*. A great attention has also been paid to microbial community within the wetland systems with respect to removal of microbial contaminants (Perkins and Hunter 2000; Thurston et al., 2001).

2.2.3.1 Removal mechanisms within constructed wetlands

It has been proved for last five decades that constructed wetlands provide an efficient method for treatment of almost all kinds of wastewater such as industrial wastewater (Kadlec et al., 2000), municipal sewage and domestic wastewater (Vymazal, 2000), leachate, dairy wastewater, livestock wastewater and distillery wastewater etc. Constructed wetlands provide an inexpensive and easily managed means of removing contaminants of three broad category such as organic matter degradation (biological and chemical), nutrients and microbial which includes biological oxygen demand, chemical oxygen demand, particulates, nitrates ammonia and phosphorus), dissolved and suspended solids and bacteria from wastewater. All the contaminants are removed thorough mechanisms shown in Table 2.2.

All the mechanisms presented in the table as above must be followed within the constructed wetland at their best level for their importance. Although constructed wetlands offer great combination of biological, chemical as well as physical process, the ecological aspects must be considered along with their biological environment associated with media and plant parts. Following is the detailed understanding about removal processes of above mentioned three categories.

Aerobic and anaerobic degradation of organic matter

The degradation within wetland system occurs due to presence of organic matter and decomposers simultaneously. The organic matter is provided from application of wastewater then simultaneously supports the growth of the bio-film (including decomposers) onto the existing medium within wetland system. The ecological conditions in constructed wetlands suggest the presence of aerobic and anaerobic bacteria within their respective zones, through which wastewater comes in contact and resulted the biochemical process.

Table 2.2

Removal mechanisms with their corresponding pollutants within constructed wetland system (Khalil, 2009)

	Processes/mechanism	**Treatment/pollutant(s)**
Physical	Sedimentation	TSS, BOD, P, TN
	Filtration	TSS, BOD, Bacteria
	Adsorption	P, TN, Bacteria
	Volatilization	Ammonia
	Crystallization	Ammonia
Chemical	Precipitation	P, TN, Heavy metals
	Adsorption	P, TN, Heavy metals
	Hydrolysis	BOD
	Oxidation/reduction	BOD, TN, Bacteria
Biological	Bacterial metabolism	BOD, TN, Bacteria
	Plant metabolism	TN, P
	Plant adsorption	TN, P
	Natural die-off	Bacteria
Ecological	Predation	Bacteria
	Food chain	Heavy metals, TN, P
	Bioaccumulation/ Biomagnification	Heavy metals
	Succession	TN, P

Transport of oxygen to the system is found due to the root and rhizomes of reeds and all other wetland plants which are hollow and contain air filled channels that are in contact with the atmosphere (Vymazal, 2005). Maximum amount of the transported oxygen is used by root and rhizomes itself for their respiration but as they are not completely air tight, some amount of present oxygen is released to the atmosphere (Brix, 1994, 1997). Biological degradation, which is measured in terms of BOD_5, is greatly influenced by the presence of oxygen and aerobic heterotrophic bacteria. Higher metabolic rate of these bacteria causes more biological degradation and in result reduces the amount of BOD_5. So far, in the aerobic zone, heterotrophic aerobic bacteria and amonifying bacteria govern the degradation of organic matter (Cooper et al., 1996). Amonifying bacteria degrade nitrogenous organic compound under aerobic conditions. In other words, oxygen availability becomes limiting factor for function of aerobic microbes. In aerobic zone, nitrifying bacteria use oxygen for their physiological need, outcompeted by heterotrophic bacteria for Oxygen (Brix, 1998).

In anaerobic zone, respiration and degradation occurs by facultative or obligate anaerobes in which higher molecular weight carbohydrates are broken down into their simpler form like dissolved organic carbon which becomes available to the microbes (Vymazal, 2005; Valiela, 1984). The anaerobic degradation occurs in multiple steps. Firstly through fermentation primary end-products are formed. Primary end-products can be fatty acids such as acetic acid, butyric acid, lactic acids, alcohol, and gases CO_2 and H_2 (Mitsch and Gosselink, 2000; Vymazal, 2005). These end-products are finally taken up by sulphate reducing and methane forming bacteria. Both of the groups importantly take part in the organic matter decomposition (Valiela, 1984; Vymazal, 1995; Vymazal, 2005). This should be noticed that the anaerobic degradation occurs at slower rate than the aerobic one.

Nitrification/denitrification for removal of nitrogen

Nitrogen, being a contaminant, works as nutrient also for all the biological species exists within the wetland system such as plants and microbes. Still its excessive amount of makes it unsuitable. In discharged effluents, nitrogenous compounds are generally measured in term of NH_4-N, NO_3-N and NO_2-N, because of: a) high biological oxygen demand, b) toxicity of unionized ammonia (NH_3) to various forms of aquatic life under certain pH condition, c) toxicity of NO_3-N and NO_2-N and their contribution to the eutrophication of lake and reservoirs (Hammer and Knight, 1994).

Hence, the removal of these matters is necessary and is automatically resulted from biochemical reaction when favorable environmental conditions occur within the system. Further studies show that nitrogen may exist in different forms such as ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-). Gaseous nitrogen may exist as dinitrogen (N_2), nitrous oxide (N_2O), nitric oxide (NO_2 and N_2O_4) and ammonia (NH_3). In a broad way, the removal of nitrogenous matter is mainly a function of nitrification/ denitrification process within the wetlands system (Vymazal, 2005). Vymazal (2007) explained transformation mechanisms of nitrogen in wetlands as below; nevertheless, the chemical processes are dependent on several environmental factors such as pH, temperature and others. Table 2.3 explains the different mechanisms and the suggested nitrogen removal rate.

Table 2.3

Removal mechanisms of nitrogenous matter and their respective rates

Process	**Transformation**	**N_2 removal rate ($gNm^{-2}\ d^{-1}$)**
Volatilization	ammonia-N (aq.) → ammonia-N (g)	2.2
Ammonification	organic-N → ammonia-N	0.004 and 0.53
Nitrification	ammonia-N → nitrite-N → nitrate-N	0.048
Nitrate-ammonification	nitrate-N → ammonia-N	
Denitrification	nitrate-N → nitrite-N → gaseous-N_2, N_2O	0.003 – 1.02
N_2 Fixation	gaseous-N_2 → ammonia-N (organic-N)	0.03 and 46.2 (per year)
Plant/microbial uptake (assimilation)	ammonia-, nitrite-, nitrate-N → organic-N	
Ammonia adsorption		
Organic nitrogen burial		
ANAMMOX (anaerobic ammonia oxidation)	ammonia-N → gaseous N_2	

Removal mechanisms for microbial contaminants

Many authors suggested different ways for removal of microbial contaminants as they have been observed during their period. The Major removal of microbial species is found to be offered by three types of processes: physical, chemical and biological, where removal of these contaminants occurs by means of interaction of these three processes through biochemical interactions. All the contaminants are removed in the wetland system but each material has its own way and fate within the wetland environment.

Physical processes involve sedimentation, ultraviolet radiation, solar irradiation, aggregation and mechanical filtration. Chemical processes involve oxidation, exposure to biocides excreted by some wetland plants and absorption by organic matter. And the biological processes for pathogen removal are antibiosis, predation by nematodes and protest, competition attack by lytic bacteria and viruses and natural die off (Gersberg et al., 1989). Scott et al. (2003) observed that the removal of bacterial indicators has been significantly affected by a filtration method especially by per-chlorination. He stated that the extended time with free chlorination has better impact on removal of bacterial community. According to Mandi et al. (1993) in sub-surface flow constructed wetlands, removal of Fecal coliform is facilitated by sedimentation, predation and death with macrophyte populations such as hyacinth.

Table 2.4

Description of different removal mechanisms for microbial contaminants occurring within constructed wetlands (Vymazal, and Kröpfelová, 2008)

Mechanism	Description
Sedimentation	Trapping pathogen in sediments
Surface adhesion	Trapping pathogens on biofilm primarily supported by emergent macrophytes
Aggregation	Natural process resulting in clumping of particles. Aggregation is enhanced in wetlands because wetland vegetation reduces water column mixing forces that act to break up aggregation.
Natural die off and competition	Exposure to hostile environmental conditions - particularly high and variable temperature, dissolved oxygen, redox and pH. For pathogens trapped in biofilm in particular, competition for resources with the natural microbiota is a factor in die-off. Part of this competition may be the excretion of substances that are toxic to pathogens and other organism
Predation	Many pathogens may be eaten by filter feeding zooplankton. Even where ingestion dose not kill the pathogen, it effectively packages the pathogen into fecal pallets that are more easily removed from the water column
UV exposure	Exposure to strong sun light and UV radiation can damage proteins and genetic material in many organisms. Enteric bacteria and viruses are most susceptible to this mechanism

Keffala and Ghrabi (2005), observed that the removal of bacterial contaminants through gravel based medium is basically done with the help of filtration and sedimentation. Molleda et al. (2008) stated that the chemical factors which contribute in the removal of microbial contaminants are oxidation reaction, absorption or exposure to plant and microbial toxins.

Additionally, vertical flow CW behaves like a percolation-infiltration system. Hence, removal of microorganisms during infiltration is facilitated through porous media by combination of straining, adsorption and inactivation.

2.3 Microbial Indicator and Pathogens in Wastewater

The presence and persistence of microbial contaminants such as bacteria, viruses and protozoa has been observed and related to the health issues studies with respect to the reuse of wastewater (Bitton, 1994; Rose, 1986; Gerba and Rose, 1990; Rose et al., 1996). These microbial contaminants in wastewater come majorly from the human excreta as these are associated with warm blooded animals (Miescer and Cabelli, 1982). Such contaminants, present in the wastewater, are categorized into two groups, indicators and pathogens. Examination of indicator organisms gives estimate about microbial contamination present in wastewater while pathogen identification is important to find out the health risks as well. Infect many times, both type of microorganisms are used to give approximation of each other if their relative quantification comes into consideration. For example, the presence of fecal indicators such as *E.coli* is usually used to predict the presence of other pathogenic bacteria (Horman et al., 2004). Also, the Fecal coliform are found to be classical indicator to determine the other pathogenic species in water samples, but sometimes, pathogens are observed in absence of Fecal coliform too. Therefore, *Salmonellae* spp. are suggested to be determine directly for pathogenic characteristics (Jillson et al., 2001). A study from past decades also observed the correlation of *Salmonella spp.* with various indicator organisms and found close relationship with *Fecal streptococcus* with *Salmonella spp.* (Morinigo et al.,1990). So far, in general, the best suggested recreation water quality indicators are *enterococci* and *E.coli* along with five other organisms *Total coliform,* Fecal coliform*, Aeromonas hydrophila, Pseudomonas aeruginosa* and *Clostridium perfringens* (Bonde, 1962). The coliform are detected in intestinal flora of mammals and other animals, and they are found with the other organisms which belong to *Escherichia* genus. Likewise, *E. coli* is found in the digestive track of human intestine and it is not usually found in other environment, that's why it is found to be a great indication as a fecal contamination. Although, *enterococci* is found to be an alternative indicator of *Total coliform* over the *E.coli* because of their greater resistance and their ability to grow in any environment such as soil, water, etc. (Vera et al., 2006 and Ryu et al., 2007). The epidemiological research found an increased link between gastro-intestinal diseases and polluted water with high concentration of these indicator bacteria (Thurston et al., 2001 Vera et al., 2006). In raw sewage the typical abundance of *Total coliform* and Fecal coliform was found with10^7 to 10^9 and 10^6 to 10^8 ml^{-1} (George et al., 2002). Therefore, their enumeration has been significant for the purposes of wastewater reclamation and reuse.

Beside all above, among the numerous type of pathogens, *Giardia* and *Cryptosporidium* are found to be most commonly isolated intestinal parasites in the domestic sewage in developing countries (Gardner et al., 2001; Rose et al., 1995, 1999). So far, it has been found that they can pass on through the various treatment processes. A study by Scott et al. (2003) investigated the removal of indicators *Total coliform* and Fecal coliform and *Enterococci* with pathogen analysis of *Giardia* and *Cryptosporidium* by three ASP based treatment plants, each was associated with disinfection process with chlorination. And FC was found below detectable level in the effluent of all three plants, while other microbial species were at detected level in effluents from all of them because the pathogens were less affected by disinfection step. In accordance of these studies, Lee et al. (2006) and Omura et al. (1989) stated that the most common ASP systems are the most efficient for removal of indicator and pathogen removal. In contrast, *Salmonella spp.* is found to survive better as compared to *E.coli* concentration in the biological processes. This might be due to development of specific survival strategies to survive when outside the host, or more generally, to survive under stress (Song et al.2008). Their survival under stress, in the water environment and in the soil was also reported by Wenfield and Groiman, (2003). In this view, a study by Koivunen et al. (2003), also indicated that high risk association with presence of *Salmonella spp.* in conventional municipal wastewater and suggested the tertiary treatment in order to reduce the health risk.

Hence, the above discussion revealed that health risk is associated with wastewater and it is needed to reduce where reuse and reclamation practices are common. Further discussion includes the removal efficiency of microbial contaminants from constructed wetlands.

2.4 Microbial Contaminants Removal from Constructed Wetland

It has been consistently reported that the microbial contaminants must be taken into consideration for their persistence in the wastewater and treated effluent as well. Environmental concerns suggested that the contamination of water by pathogens cannot be excluded while they pose a risk to public health (Hagendrof et al., 2000). Various studies investigated the removal of indicator organisms (Perkins and hunter 2000; kadlec et al., 2000; Langergraber and Haberl, 2001; Hench et al., 2003) and very few consider the pathogen estimation through the treatment wetland. The investigation of these microbial contaminants gives a better understanding about the treatment efficiency of the process in terms of sanitization. Although much research has been carried out on removal of microbial species, the information regarding reduction in number of pathogen in STP with constructed wetland as a tertiary treatment system is still limited. In studies of microbial removal, the choice of Fecal coliform and *Enterococci* as indicator organism is basically due to two main reasons: it is simple and economical, and secondly their investigation prevails information related to presence and behavior of the principal human pathogen present in the wastewater (Evanson and Ambrose, 2006; Vera et al., 2006; Orosz-Coghlan et al., 2006; Sleytr et al., 2007; Payment and Franco, 1993). There are many studies that emphasize the high efficiency of constructed wetlands for different indicators and pathogens. Although the role of these system has been investigated from the past decades in this view, where, Gersberg et al. (1989) and hill and Sobsey (2001) reported almost 96% reduction of pathogen (*Salmonella spp.*) through sub-surface CWs. A study by Gracia et al. (2003) observed the removal of Fecal coliform and *Somatic coliphages* from constructed wetland and found it an efficient system via inactivation mechanism. Within the scope of this research, the coming discussion accounted some of the reported theories along with important points of respective studies as follows.

Hench et al. (2003) performed a study on subsurface constructed wetland with detention time of 6- 8 days and observed that the removal of microbial contaminants can be achieved >99% for Fecal coliform and *Enterococci*, in contrast, pathogen removal was found to 1.5 or 2.2 log reduction. This study favors the presence of vegetation for the improvement of removal efficiency and suggested to not to use CWs as sole treatment process. In fact, it was also recommended the need for incorporation of disinfection step for achievement of permissible limits for *Total coliform*.

Sleytr et al. (2007) concluded that the removal of microbial contaminants by vertical flow constructed wetlands could be achieved by 2.05 logs, 2.85 logs, 4.30 logs, 4.35 logs and 4.80 logs for direct count, HPC, *Total coliform, E.coli* and for *Enterococci* respectively. There was no significant difference was observed in planted and unplanted wetlands except for *Enterococci*. The lower bacterial removal in two of 10 CW units was due to greater grain size of soil. It has also been observed that maximum reduction occurs within 10-20cm layer when sampling has been done at different depths of each of the CW. In the present study, the municipal wastewater was pre-treated mechanically.

A study by Molleda et al. (2008) found the seasonal variations in the removal of pathogens through CWs. In this experiment CWs get municipal wastewater after pre-treatment with lagoon and found that the best removal of Fecal coliform was in autumn with 3.18 log units at 13 day HRT. This study found a correlation between the presence of ammonia and enteric bacteria while no positive and significant correlation was observed in temperature and removal of microbes.

Song et al. (2009) observed the removal of *Fecal* and *Total coliform*, *E.coli* and *Salmonella spp.* and *C. perfringens* by 84%, 98.3%, 90.9%, 66.9%, 24.1% respectively. The system showed better removal for indicators in comparison to pathogens. When results were compared with Secondary treatment system which is A_2O (anoxic, anaerobic and oxic chambers), constructed wetlands are found to be less effective. Even the no. of Fecal coliform is found to be increased in effluent of CW than in the influent. This has been reasoned by the increased amount or microbes in influent than the effluent of secondary treatment occurred in the path between CW and A_2O process.

Torrens et al. (2009) conducted a study on VFCW with *Phragmites australis* and found out the removal for bacterial indicator and viral indicators and their relation to HRT which was confirmed by performing tracer study with NaCl. In the experiment, the inlet concentration was low around 10^4 or 10^5 CFU/100 ml and the removal for FC and *E.coli* was 0.5 to 1.5 log reduction only. And the results were found to be similar to other studies in which the hydraulic loading was same (50-130 cm/d). When results were compared with studies which showed higher removal, the reason was the lower hydraulic loading.

Keffala and Ghrabi (2005), found 90% reduction for bacterial contaminants. Rivera et al. (1997) has reported high removal (3 log or more) of FC because of influent of high concentration of FC (6-11 log unit/100 ml) through tertiary reed beds while treating slaughter-house wastewater.

So far, the above mention studies help to draw a conclusion that the constructed wetland are highly efficient system to reduce the microbial contaminants from wastewater in order to reduce the associated health risk but the performance efficiency of these system could not be expected at optimum level. There are certain factors associated with the system that may create variations in system's efficiency which are already discussed in previous sections: presence or absence of vegetation, types of vegetation, hydraulic residence time, hydraulic loading and grain size of supporting medium etc.

2.5 Summary of the Chapter: Research Gap and Framing of the Proposed Research

The review of the study suggested that the constructed wetlands are best suitable as well as efficient system for removal of contaminants from almost every type of wastewater. Hence, where the secondary effluent has been found to contain high concentration of pathogenic microorganisms, and disinfection is needed in reclamation processes with wastewater, constructed wetlands can be employed (Asano and Levine, 1998). Comparatively, in subsurface flow the contact area of water with bacteria and substrate is much bigger than surface flow construed wetlands (Sleytr et al., 2007). The literature available has a focus with regarding removal of pollutants of all three categories: physical, chemical and biological/microbial pollutants from constructed wetlands. Contaminated water is usually found to be discharged and posing health risk to public domain, which should not be excluded (Hagendrof et al., 2000). Mostly they are concentrated with primarily treated wastewater while limitation/reduction of microbial contaminants is investigated. Limited literature is available for semi-arid Indian climatic conditions that focusing on final effluent concentration of microbial contaminants which should meet the standard limits, prescribed for specific reuse, when the lower bacterial load (as in secondary treated effluents) is applied to the system. Especially, lack of information exists for their (constructed wetlands) efficacy and suitability while using under semi-arid conditions prevailing in India, to achieve bacterial load within the standard limits suggested by WHO i.e. 1000MPN/100 ml. Most of the studies, discussed in subsection of removal of microbial contaminants, are only give information about removal from influent and effluent concentration but lest knowledge has been shared about their microbiota present within the wetland system.

The above detailed literature review has identified the following key aspects with respect to this study: 1) To address the proposed aim of the study, tertiary treatment step could be employed using constructed wetlands in order to limit the microbial concentration under consideration of subsiding the effects related to cost of the system and environmental issues associated with other treatment technology options for the same. 2) To propose the system to be applicable on field level for research as well as to the government bodies to be taken into consideration, certain key aspects must be taken into account during the research formulation. Due to constraints related to land availability in developing countries the favorable flow regime vertical flow regime for the technology should be of choice using the very basic components such as gravels and sand as media with ease of availability. Whereas the selection of species for vegetation in constructed wetlands should be supportive with the prevailing climatic conditions for the objectives, so far, the most commonly used plant species *Phragmites australis* and *Typha latifolia*, and recently introduced *Canna indica* can be selected based on their proved sustainability for performance of the system.

Chapter 3
Materials & Methods

3.1 Introduction

The chapter describes in detail the experimental setup and research methodologies supporting the proposed study. Starting with the literature as a basis for selecting the various experimental components viz. type of constructed wetland, flow regime, vegetation and media type and configuration, this chapter explains the specifications of those components used in the experimental set-up along with the site description for the proposed study and brief explanation of the retention time within the wetland system. Further, this chapter deals with research methodologies that have been adopted to accomplish previously objectives of the present study including the methods used to collect information and data requirements for the study and their respective data sources and the associated sampling strategies are well documented.

3.2 Literature Basis for Selection of Components of the Experimental Setup

For developing countries like India where cost is a major consideration constructed wetland system was suitably chosen due to its low cost and easy maintenance and operation. The system attracts wildlife and appears aesthetic due to the vegetation (which lacks in conventional system) and facilitates combination of physical, chemical and biological processes in one system without any side effects. Subsurface flow was selected as there was no direct contact with environment and it leads to fewer problems of bad odor and insect proliferation. Vertical up-flow regime was chosen as it provides more and uniform root distribution, better contact area to water root zone and biofilm and less land area requirement. In vertical up-flow regime, lower retention time is effective with dominant role of filtration and homogeneous distribution of bacteria is observed at different depths (Arias et al., 2003).

The various components of the experimental setup were based on certain reported theories. Media configuration was based on locally available material which would not increase the cost of the system. Therefore, as reported in chapter 2, gravels of different sized were selected and used for the study. Precisely, it has been suggested to use gravels and sand; gravels support the vegetation, provide aeration throughout the system (especially in case of vertical flow) and allow bio-film to grow, and sand being the finer material, is efficient in removal of microbial contaminants (Gracia et al., 2003; Sleytr et. al., 2007). For vegetation *Phragmites australis* and *Typha latifolia* were chosen as they are commonly used plant species for wetland establishment while *Canna indica* was selected because it is an Indian shoot. Additionally, *Canna indica* was found to be compatible for Indian conditions (Jayakumar and Dandigi 2002; 2003).The depth of the system was chosen on the basis of reported literature. Study suggests 30-50cm media depth being beneficial for better aeration conditions (Srinivasan et al., 2000). In support of the selection criteria for depth of the treatment units, it has been well documented that root length of commonly used plant usually does not exceeds 80cm, including some studies recommending a depth of 100cm (Brix and Arias 2005; Prochaska and Zouboulis, 2009).

3.3 Materials

3.3.1 Study site and source of secondary treated wastewater

Experimental setup was established at Civil Engineering Department, Malaviya National Institute of Technology, Jaipur, India, where four constructed wetland units were used to improve the quality of secondary treated wastewater. The secondary treated wastewater was sourced from the sewage treatment plant located in south Jaipur throughout the study period. The effluent from the field was brought to ensure actual effluent quality of STPs. The treatment plant with capacity 62.5 MLD municipal wastewater was based on Activated Sludge Process. The effluent of treatment plant was disposed after secondary clarifier and was used to irrigate surrounding crop land. Such effluent was sourced via submersible pump into jerry cans of 20 liters capacity and was carried every third day to the study site, where it was finally kept in a feeding tank of 100 liters capacity. The tank was used to feed all the connected constructed wetland units throughout the experimental period. Each treatment unit was separately connected to the feeding tank via peristaltic pump (*Make: ENERTECH-ENPD Victor 100; Range: 0.3 - 450 ml per minute*) and silicon piping of 2mm inner diameter and 4mm outer diameter. The connecting ports on the feeding tank were placed at 10cm above from the bottom line in order to prevent the entry of the settled particles from the tank into the wetland units. Effluent collection pots were placed under the outlet port for each of the wetland unit. The study site was surrounded with an iron cage to keep it as separate area during the research period.

3.3.2 Pilot scale vertical up-flow constructed wetlands: experimental setup

Four pilot scale vertical up-flow constructed wetland (VUFCW) units were established. Initially, only three units, UN-ph, UN-cn and UN-ct, were constructed which were similar in terms of bed media configuration but different in terms of plantation (Figure 3.1a). Unit UN-cn-sd was developed in the later course of the study on the basis of observed results from first three units to improvise the performance of the constructed wetland units for removal of microbial contaminants. Unit UN-cn-sd had same plantation as UN-cn but was different in terms of bed media configuration. The unit was improvised using sand layer of 15cm with finer gradation than other units (Figure 3.1b). All the containers were placed outside the Environmental Laboratory of Civil Engineering Department at MNIT Jaipur. All wetland units were located in the same open area where direct sunlight comes for some part of the day and absolute day light remains throughout the day.

All the treatment units were established in trapezoid shaped *Sintex* tank. Their trapezoid structured can be observed in figures 3.2-3.5. The width is different at different heights of the container. Width is increasing up the height of the container starting with 25cm at the bottom to 27cm at the middle point and all the way up to 29cm at the top surface. The bottom length of each unit was smaller than the surface length by 4cm (29-25cm). The empty volume of each of the treatment unit was 51,123cm^3/51.123L. The connection of the feeding tank and treatment units were established through silicon pipes attached with separated outlet ports of feeding tank to inlet point of each treatment unit. The treatment units were installed with inlet port at the bottom and outlet port at the top. A steel sieve was kept at 12cm (H1) above from the bottom in each of the treatment unit, to separate the inlet chamber from treatment bed and it also helped in uniform distribution of water throughout the treatment bed.

The treated wastewater first flows into the inlet chamber via inlet valve. It then rises upwards to the treatment bed to allow for continuous flow and is finally collected from outlet ports into the effluent pots. Figure 3.1 shows the transverse section of media distribution for all four constructed wetland units and Table 3.1 gives the detailed design configuration of the experimental set up for all the constructed wetland units.

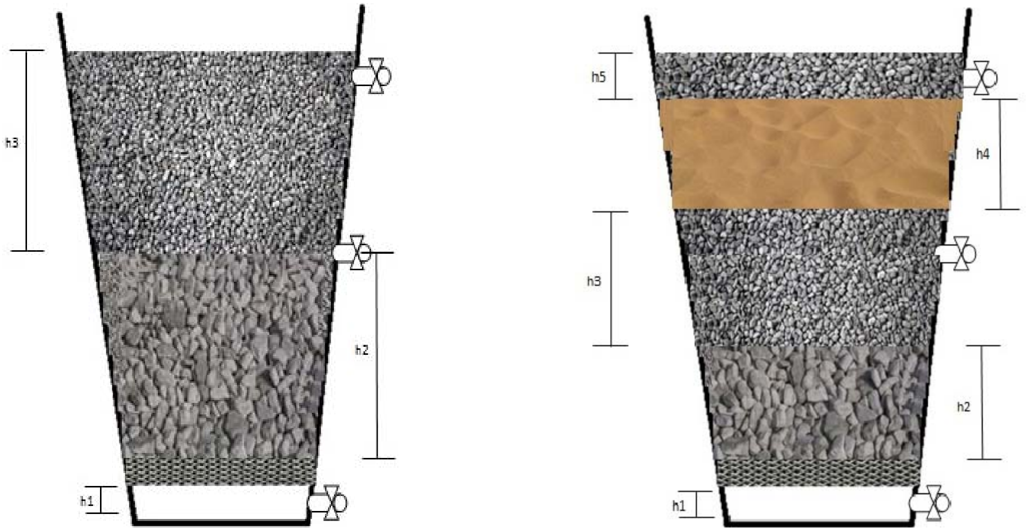

Fig. 3.1. Transverse cross section of two different types of constructed wetland units (a) Distribution of media for UN-ph, UN-cn, UN-ct (b) Distribution of media in UN-cn-sd

Table 3.1

Design configuration of experimental setup

Constructed wetland unit	Height and type of media				
	H1	H2	H3	H4	H5
UN-ph Planted with *Phragmites australis*	Inlet chamber	Media – Gravel Size - 16-20mm Height - 29.2cm	Media – Gravel Size - 8-12mm Height - 22.9cm	-	-
UN-cn Planted with *Canna indica*	Inlet chamber	Media – Gravel Size - 16-20mm Height - 29.2cm	Media – Gravel Size - 8-12mm Height - 22.9cm	-	-
UN-ct Control unit No plantation	Inlet chamber	Media – Gravel Size - 16-20mm Height - 29.2cm	Media – Gravel Size - 8-12mm Height - 22.9cm	-	-
UN-cn-sd Planted with *Canna indica* with sand layer	Inlet chamber	Media – Gravel Size - 16-20mm Height - 15cm	Media – Gravel Size - 8-12mm Height - 25cm	Media – Sand Size - 1-2mm Height - 15cm	Media – Gravel Size - 8-12mm Height - 1-2cm

3.3.3 Hydraulic retention time (HRT)

The system was operated at flow rate of 6.8 l/day with continuous loading to ensure 2-3 days of hydraulic retention time (HRT) for each treatment unit. The HRT was selected based on the literature review (Gracia et al., 2003; Ghosh and Gopal, 2010). The HRT was confirmed in terms of the actual volume present in each unit which is equal to the void volume of the unit. The void volume of each of the treatment unit was measured to be the amount of water that covers the surface of media completely when introduced from the inlet port at the bottom and was found to be in the range of 18-20 l for each of the treatment unit. Hence, the HRT is calculated, based on the flow rate of 6.8 l/day, to be in the range of 2-3 days, as suggested in the literature.

HRT (H) =Volume (V) /Q (Flow rate) = 18/6.8 (2.64 days) to 20/6.8 (2.94 days)

3.4 Research Methodology

The research purpose was explored and designed to investigate the viability and adaptability of vertical up-flow constructed wetland as a post treatment technology in semi-arid Indian climatic conditions. The development of best suitable combination of vegetation and media for constructed wetland and assessment of its efficiency for covering broad applications was then formulized. The research strategy was developed and designed keeping in mind the very basic features of constructed wetland and their relationship with various aspects of water reuse and reclamation strategies.

3.4.1 The methodological framework

The logical stepwise methodologies were adopted during the research study to accomplish the key objectives of the study that were formulated within the aim of the study. The data and information required with respect to each objective was collected to perform various analysis. All such information is collectively well defined in Table 3.2. Broadly, data set was categorized into primary and secondary data. The primary data was generated through performing experiments during various stages of the research study. The secondary data was in the form of literature review and electronic sources related to constructed wetland systems. The data collection for the study was divided into different stages viz. acclimatization and establishment stage of constructed wetlands, background analysis of each system i.e. removal of physico-chemical contaminants and final and major stage was analysis of constructed wetland units for microbial contaminants removal.

Table 3.2

Research objectives and data information - needs, sources, collection method and analysis

	Research Objectives	**Data Information**		
		Data/information required	Data Sources (primary/secondary)	Data analysis
1.	To assess the survival and growth of locally available plant species in secondary treated wastewater for semi-arid climatic conditions	Detailed information related to behavior and growth pattern of plant species using WW in CWs	Electronic sources and acclimatization period: primary as well as secondary data through experimental trials	Visual observations
2.	To analyze the performance of laboratory scale experimental constructed wetlands with reference to removal of organics, nutrients and microbial contaminants from secondary treated wastewater	Detailed knowledge about the role of physical and chemical parameters with respect to VFCW, i.e. to get functional background information of any of the system and performance efficiency for removal of all three contaminants categories	Electronic sources and experiments at different stages of the research study period: accurate primary data that should be reproducible	Comparing observed data
3.	To identify more suitable plant species for removal of contaminants from secondary treated effluent	Extensive observation of growth patterns of plant species and knowledge of their contribution for removal efficiency, corresponding loading concentrations	Primary as well as secondary data to verify the observations for particular species	Literature review and experimental observations
4.	To assess the role of media on performance of laboratory scale constructed wetland units	Comparative data for the concerned parameters using different media configurations	Primary as well as secondary data to verify the observations for particular media	Data analysis using statistical tool
5.	To identify best performing constructed wetland with achieving the standard limits for coliform	Compare and analysis of data observed during the whole research study period	Primary as well as secondary data to verify the observations for particular CW	Data analysis using statistical tool

3.4.2 Steps or stages taken during the research period

Starting with the collection of literature and key objective formulations, research problem was identified. A startup phase was initiated with the establishment of constructed wetland units; specifications of each treatment unit is already described in the material section of this chapter. After the completion of the start-up phase or acclimatization period, experimental analysis was initiated. The experimental analysis was designed into different stages starting with the physico-chemical parameters study of the three CW units (UN-ph, UN-cn and UN-ct) followed by the analysis for microbial contaminants using influent and effluent from inlet and outlet ports respectively. It has already been mentioned in the previous section that on the basis of the results obtained from the first three units (UN-ph, UN-cn and UN-ct) one another unit UN-cn-sd was established which incorporated all the basic features of the UN-cn but improvised in terms of the media and its gradations.

As per the reported literature, the efficiency of the wetland system improves when a finer media is used. Therefore, after identifying that the unit planted with *Canna* (UN-cn) was more efficient than the other two units (UN-ph and UN-ct), the next objective was to further explore if the removal efficiency of this unit can be improved by improvising on the media. Hence, a layer of finer material (sand) was introduced in the system. In order to maintain the total height of the system as earlier, after introduction of the sand layer the thickness of the coarse bottom gravels (16-20mm) was decreased from 29.2cm to 15cm. It was considered necessary to keep the gravel layer for supporting the sand layer and also as a barrier between influent system and sand. The complete removal of gravels would have had operational challenges. Among the coarse (16-20mm) and finer (8-12mm) gravels layer, the coarse layer was reduced for a smooth gradation from finer to coarse material considering efficiency has been positively affected by the finer material. So, this unit was analyzed separately in the later course of the experimental stages for microbial parameters followed by the background analysis via physico-chemical analysis using influent and effluent from inlet and outlet ports respectively. Further, to confirm the role of finer material (sand) in the microbial removal in UN-cn-sd, the unit with sand was further analyzed at middle point also. The concentration change from bottom inlet to the middle port will give the approximate reduction without the sand layer and the concentration change from middle to the top outlet port will determine the effect of presence of sand layer present in the upper part of the constructed wetland.

3.4.3 Startup & acclimatization period: establishment of constructed wetland units

Once the various components of the wetland units were appropriately placed and well-connected, the vegetation and the development of biofilm within the system occurred as a part of the acclimatization process. Acclimatization stage is the time within which the system generates its functionality and prepare itself to behave as a treatment system. The period covers the initial treatment stage of the constructed wetland units.

Initially, three constructed wetland units were established using three different plant species. *Tyhpa latifolia* was sourced from a marsh in Alwar, a nearby located city, while *Phragmites australis* and *Canna indica* were sourced within Jaipur from well-established wetland system and MNIT campus nursery, respectively. Before plantation, the root part of the three species were thoroughly washed using flowing tap water in order to remove sand particles. The wet plantlets of these three species were planted in the gravel media of the constructed wetland units with 10cm root part immersed in the media. Five small plantlets with height of 20-25cm of each plant species were planted to acclimatize as well as to grow in the prevailing climatic conditions and monitored regularly for survival and growth for 2-3 months (Figure 3.2, 3.5). Acclimatization of all units were achieved primarily through the use of diluted wastewater in the ratio of 7:3 with the tap water. The dilution was decreased on weekly basis and finally the system was fed with pure secondary treated wastewater which was taken from the STP (sewage treatment plant) in Jaipur. Such mixing of wastewater was done in order to allow vegetation and microorganism to acclimatize within the system's environmental conditions. The growth period and maturation stages of all four constructed wetland unit are shown in Figure 3.3, 3.4 and 3.5.

3.4.4 Growth conditions for all constructed wetland units

The experimental research is conducted in Civil Engineering department at MNIT Jaipur, Rajasthan, India. Jaipur is located at latitude 26°1'36" North and longitude 75°4' 32" East and is situated on the eastern boundary of Thar desert- a semi-arid land.

All the units were planted in the month of April. The historical average minimum and maximum temperatures during April are 21.8°C and 37.2°C respectively which is suitable for the plants growth - optimum temperature for growth of *Phragmites australis* is 30-35°C, for *Canna indica* is 12-32°C and for *Typha latifolia* is ~35°C.

After planting the plantlets, growth pattern was observed for each plant species in terms of their propagation. Once they started to propagate the plant species were allowed to acclimatize for the period of 2-3 months till the month of July.

The annual year is broadly divided into four seasons namely - the winter season from mid-December to mid-February, summer or hot weather season from March to May, monsoon season spread from end of June to mid-September, and October and November are known as transit period or post monsoon period.

The summer season is customary called hot weather season in Jaipur. The city and its suburbs experience dry and hot weather conditions during these months. The maximum temperatures hover at 40°C to 47°C in May and heat wave prevails for days in the season, when day temperature rises few degrees above normal. Generally, the average monthly wind speed varies in between 3-10kmph during the year. But in summer, there are dust storms, dust-raising winds prevailing and wind speed reaches up to 10kmph in the month of May. Dry and hot air called 'lu' (a Hindi word) paralyze the day time activities on many occasions. Rising day temperatures and convective weather phenomena viz. thunderstorms, dust storms, hailstorms, dust-devils and squalls are major characteristics during the 2nd half of this season. Dust storms are prominent weather vagaries of this season and are called "Andhi" in lingua franca.

The lowest humidity is observed in the month of April. May onward humidity picks up and increases gradually to have its highest values in the month of August. Monsoon sets over Jaipur in the last week of June. From July onwards the rainy season starts and the monsoon winds penetrate this region in the months of August and September. On outset the average mean rainfall is ~70mm in June and in July it reaches ~221mm, in August it is ~195mm and at the end it decreases to ~71mm in September. July and August are the rainiest months. The winter minimum temperatures remain about 4–9°C and sometimes even fall below zero deg. or so when chilly wind (northerly) blow from Himalayan region. Mist and fog occur in the morning hours after passage of western disturbances.

(a) (b)

Fig. 3.2. Acclimatization and establishment stage of experimental set up: (a) feeding tank connected with two planted units: one with *Canna indica* and *Phragmites australis* and one unplanted unit (control) (b) five plantlets of *Canna indica* at sowing time

Fig. 3.3. Growth period of initially developed planted units with *Canna indica* (left) and *Phragmites australis* (right)

(a)

(b)

(c)

Fig. 3.4. Mature stage of the all three constructed wetland units (UN-ph, UN-cn and UN-ct) during the monitoring period (a) *Canna indica* (b) *Phragmites australis* (c) Unvegetated control unit

(a) (b)

Fig. 3.5. Unit with different media configuration and gradation planted with *Canna indica* (UN-cn-sd) (a) at initial development stage and (b) at maturation stage

3.4.5 Sampling and experimental analysis

The experimental analysis was carried out wherein physico-chemical analysis and microbiological analysis was performed separately. Initially, only first three units were analyzed and fourth unit was analyzed in the later course of the experimental study. The analysis performed during acclimatization period was not considered for final results of this proposed study as they do not reflect the actual performance of the proposed wetland units. Macrophyte or plant species were harvested once a year during the month of July. Water samples of approximately 100ml were collected from influent and effluent to evaluate the performance analysis of all four different units. The influent samples were collected from inlet port present at the bottom of the units which opens into inlet chamber while the effluent samples were collected from the outlet port placed at the top of the units where the treated waters flows out. Only one inlet sample was taken from the bottom of the storage tank and the same was applied to all four units while outlet samples were taken from each treatment unit separately. Such sampling was done every third day during the experimental analysis period. The sampling location remained the same throughout the experimental analysis in order to ensure the consistency in the results. The sample collection was made using acid pre-washed PVC bottles while for microbiological analysis sample bottles were prepared by sterilization through autoclave before each sampling.

Sampling from inlet and outlet port was done for physico-chemical as well as microbial contaminants for each of the treatment unit. UN-cn-sd was also observed at middle point sampling in order to know the effect of finer sand material on indicator organisms. Physico-chemical analysis gives background functional information about the treatment system that could support the removal mechanism of microbial contaminants.

For microbiological analysis three species - *Total coliform* (being the most common indicator), *Fecal streptococci* (more resistant to environmental stress and known as secondary indicator) and *Salmonella typhi* (might present in the absence of indicators, Jillson et al., 2001), were used. The microbiological analysis of indicators organisms (*Total coliform* and *Fecal streptococci*) were performed using multiple-tube fermentation according to the method given in APHA (1999; 9221B and 9330B) and the enumeration of pathogenic specie (*Salmonella typhi)* was performed as suggested by Pant and Mittal (2008). Microbiological analysis was performed within 2 hours of sampling.

All physico-chemical parameters were analyzed according to methods given by APHA 1999. For physical parameter analysis pH and DO were measured while chemical parameter analysis was made through COD, NH_3-N, TKN and NO_3^--N. All physico-chemical parameters were analyzed according to methods given by APHA (1999). pH and DO was measured using electrode/probe, COD through Closed Reflux-Colorimetric Method, NH_3-N through Phenate Method, Total Kjeldahl Nitrogen (TKN) through Macro Kjeldahl Method and NO_3^--N was done by ultraviolet spectrophotometric screening method. These physico-chemical analysis were performed within 12 hours of sampling.

3.4.6 Bacterial consortia analysis around plant root & media layers: biofilm study

Bacterial enumeration around media layers of all four wetland units as well as three rhizosphere from the planted units was accomplished using biochemical tests followed by culture-dependent method.

To analyze the bacterial consortia around the filter media three major steps were followed:

1. Dismantling of each constructed wetland unit
2. Collection, transportation and storage of media samples from different sites of each unit
3. Isolation and identification of bacterial species through culturing and biochemical testing of the suspected bacterial species

Dismantling was done using sterilized gloves and each media layer was separately removed from each constructed wetland unit manually. During the process, each type of media layer (gravel and sand) was separated from around the outlet port, middle port and inlet port corresponding to the presence of microbial species at top of the treatment bed, middle zone and inner most part of the bed of each treatment unit. During separation of media layers, all three rhizosphere were also separated carefully from the planted units. Three samples were from rhizosphere of two plant species from three units, rest of the ten samples were from corresponding media layers of four constructed wetland units. UN-ph was analyzed only for its rhizosphere since it has the same media distribution as in UN-cn and UN-ct. Total thirteen samples were analyzed for bacterial diversity and their relative abundance or Phylotypic richness within the four constructed wetland units.

For the collection and transportation of all media and rhizosphere samples, sterile autoclave suitable bags were used and stored immediately in the icebox. The box containing all such samples was transported to the laboratory at B. Lal Institute of Technology, Jaipur and further experiments for enumeration of bacterial consortia were performed. The enumeration of bacteria consortia was carried out in three stages which included isolation of microbes by serial dilution method, morphological identification by Gram Staining method and final identification of species through biochemical testing (depending upon suspected species).

For isolation of species, 10g of each sample was taken and mixed with saline solution on magnetic stirrer for 15 minutes to extract the microbial assemblage from the samples. Serial dilution was carried using NaCl saline and sample dilutions were prepared. One ml of each dilution was then poured onto sterilized plates of NA, YEAMA, ENDO, XLD and ASHBY. Plates were then incubated in inverted position for 24 to 48 hours at 37^{o}C. On completion of the incubation period, microbial species were identified both morphologically (shape, gram staining and motility) and biochemically (Indole Production, Simmon Citrate Utilization test, Methyl Red-Voges-Proskauer test (MR-VP), Triple Sugar Iron (TSI) utilization, Oxidase Production, Catalase Production).

3.5 Data Analysis

The influent and effluent concentrations of physico-chemical parameters were measured in milligram per liter (mg/l) while the concentration of microbial species were observed as MPN (most probable number) per 100 milliliter (MPN/100ml) according to the table 9221: IV in APHA 1999. Such MPN values were converted to log scale. The performance efficiency of the proposed system was calculated in terms of concentration removal observed for all parameters. The percentage removal for physico-chemical parameters was calculated as below:

Percentage removal = $(Ci - Co)/Ci \times 100$

(Ci and Co represent the influent concentration and effluent concentration respectively)

While the log removal for microbial removal was calculated as: $\log_{10}(Ci) - \log_{10}(Co)$. Further, a statistical analysis was performed using two tailed t-test to determine the significance level at $p<0.05$ for different sets of experimental data.

The analysis of bacterial assemblages of all constructed wetland units was defined in terms of percentage-richness distribution and diversity. The distribution and diversity was calculated by first categorizing the observed microbial species into three different phyla of Proteobacteria, Firmicutes and Actinobacteria. Phylotypic richness was calculated as a total sum of percentage occurrence of species belonging to a specific phylum and diversity was calculated in terms of number of different species found in given samples (Garbeva et. Al., 2004).

Once the experimental setup was established and plant species were able to successfully acclimatize to the environment, all constructed wetland units were analyzed for physico-chemical with focus on microbiological parameters. The observed data results were compared with the reported literature and statistically analyzed using t-test ($p<0.05$).

Chapter 4
Results and Discussion

4.1 Introduction

In the previous chapters the proposed research problem has been defined, the historical background in the form of literature review has been studied and current scenario along with the knowledge gap was identified. The experimental setup including experimental site, design and operational aspects of the system were also discussed in detail. This chapter presents the results of well-established constructed wetland units obtained throughout the research study and discusses the outcomes based on inferences from the observed results. Experimental analysis of the results is also supported by statistical analysis. The outcome reveals the plant species most suitable for semi-arid climatic conditions as well as the best suitable combination of vegetation and media within the constructed wetland system. The results are majorly focused on removal of microbial species from secondary treated wastewater whereas the physico-chemical analysis is performed as a background analysis.

The study period of the research is defined in weeks in table 4.1 which presents the time spent on all the activities performed during the study period. The total study period was around 93 weeks (~21 months) which includes 50 weeks of analysis for first three units (rows 1-3) and remaining 43 weeks for the analysis of fourth unit (rows 4-7). Total of 70 weeks were dedicated towards experimental and sampling analysis (rows 2-3 & 5-7) and rest of the time was the part of adaptation/ acclimatization period (rows 1 & 4).

The three units UN-ph, UN-cn and UN-ct were planted first. The adaptation period for these units was 12 weeks after which the sampling and testing was done for a period of 38 weeks (13 weeks of physico-chemical analysis followed by 25 weeks of microbial analysis). The fourth unit was then developed and was allowed to acclimatize for a period of 11 weeks. After the adaptation period, the sampling and testing was done for a period of 32 weeks (9 weeks of physico-chemical analysis, followed by 20 weeks of microbial analysis, followed by 3 weeks of middle point analysis).

Throughout the study the data was collected such that the time lag between the inlet sample and the corresponding outlet sample was maintained at a difference of three days in order to allow the inlet to reach the outlet as per the HRT of 3 days. Also, throughout the study the samples for physico-chemical analysis were taken at a frequency of twice in a week and for microbial analysis the frequency was one sample per week.

Table 4.1

Time schedule for various activities involved throughout the study period for all the units. All the numbers here are in weeks

Activity		Influent	Effluent				Middle pt.
			UN-ph	UN-cn	UN-ct	UN-cn-sd	UN-cn-sd
1.	Acclimatization Period	-	12	12	12	-	-
2.	Physico-chemical analysis	13	9	9	9	-	-
3.	Microbial analysis	25	17*	20	12#	-	-
4.	Acclimatization Period	-	-	-	-	11	-
5.	Physico-chemical analysis	9	-	-	-	9	-
6.	Microbial analysis	20	-	-	-	20	-
7.	Middle Point Analysis	3	-	-	-	3	3

* Out of 20 week sampling, 17 times the results were obtained
# Out of 17 week sampling, 12 times the results were obtained
Results were not obtained sometimes due to the experimental error or due to technical failures

4.2 Constructed Wetland Vegetation

Many plant species are being in use for establishment of constructed wetlands worldwide; literature review in Chapter 2 precisely covers the role and significance of each. So far, the most commonly used plant species, *Typha latifolia, Phragmites australis* and *Canna indica* were chosen on the basis of availability, suitability and climatic conditions. This section defines these plantations that were considered for the proposed study and their analysis during the acclimatization period.

The three plant species *Typha latifolia, Phragmites australis* and *Canna indica* were monitored for survival and growth for four weeks; they were monitored for their general appearance, growth pattern and propagation in the constructed wetland units. During the first three-four weeks of plantation, deterioration was observed for *Typha latifolia* whereas *Canna indica* showed various buds and started to propagate rapidly. *Typha latifolia* was planted again in a separate pot to acclimatize in the surroundings but failed to survive. The most possible reason for *Typha latifolia* deterioration could be the low organic loading application through secondary treated wastewater whereas plantlets of *Typha* were soured from the marshy area which was rich in organic contents.

Canna indica grew well throughout the study period except for a brief dormant growth period during peak summer season (May to June). The *Canna* plant species grew initially at a height rate of ~7-8cm per week and it attained a height of 50-55cm after four weeks of plantation and first flower was observed after six months of plantation. Finally after three months *Canna* plant achieved a height of 85cm (average of all plantlets). The growth in leaf count of *Canna* plant species was on average ~3 leaves per week with minimum and maximum leaf count of 3 and 7, respectively, within a single plantlet. The per week leaf area increment was not taken into account. The propagation was observed in terms of growth of new plantlets from the existing one. The five *Canna* plantlets that were planted during the time of establishment of the experimental setup, propagated to a total of 15-17 plantlets after three months. Thus, it was found that the *Canna* species grows rapidly initially but after it reaches a certain height its growth rate slows down and propagation gets faster. No sign of full loss was observed for already standing strands and observed deterioration was only in terms of wilting of leaves and their shed off. It was observed that this macrophyte was much harder and once they had grown enough they were able to maintain themselves well from harsh environmental conditions of strong winds and intense sun light. Its wide leaves covered the entire surface of the wetland units with high intensity. Its steady and vigorous growth indicates that it has high tolerance and survival capacity in semi-arid conditions prevailing in Rajasthan, India.

Phragmites australis plantlets showed positive response in terms of propagation and growth after four weeks of plantation. Although *Phragmites australis* took time to show some visual signs of growth as compared to *Canna indica* but started growing well with high enough strands. Its (*Phragmites australis*) height increased at a slower rate of ~4cm per week; in few weeks even no increment was observed. After a period of four weeks it attained a height of 35-40cm. But, once it started to grow vertically it attained a height even higher than the *Canna* plant species and after three months reached a height of 115cm (average of all plantlets). The growth in leaf count increased more rapidly in case of *Phragmites* with ~6-8 leaves per week. The five plantlets that were planted during the time of establishment of the experimental setup, propagated to a total of 30-35 plantlets after three months.

On the basis of the observed responses of three different plant species, two of them *Canna indica* and *Phragmites australis* were selected as best suitable plant species to pursue with constructed wetland establishment in prevailing climatic conditions. Further experiments were carried out through application of secondary treated wastewater on constructed wetland units with the aim to reduce the microbial load in terms of reduction of *Total coliform (TC), Fecal streptococci (FS)* and *Salmonella typhi (S.typhi).*

4.3 Characteristics of Secondary Treated Wastewater

Although, the focus of the proposed study has been the reduction of microbial contaminants from secondary treated wastewater, but still it's physical and chemical characteristics must be monitored to ensure the quality of influent being used for the treatment wetland. Such information helps to infer efficacy of the treatment system for the corresponding parameters and helps in deciding design parameters and operational criteria for the constructed wetland units. However, all parameters are of equal importance when the concern is only to know about the basic quality of wastewater, regardless of the focus of research problem.

Within the abovementioned context, the secondary treated wastewater sourced from sewage treatment plant was examined both, before the treatment process started as well as during the treatment process, for all three category of parameters - physical (pH, DO), chemical (COD, TKN, NH_3-N, NO_3^--N) and biological (*TC, FS, S.typhi*). The treated wastewater was fed to the treatment units throughout the experimental period and worked as influent for constructed wetland units. The quality analysis of secondary treated wastewater was monitored at the inlet of constructed wetlands throughout the monitoring period of the study. A summary of results for all the parameters of the secondary treated wastewater is presented in Table 4.2 (a, b).

Table 4.2

(a) Physico-chemical characteristics of secondary treated wastewater used to feed as influent for four constructed wetland units (n=29)

Parameter	pH	DO*	COD*	TKN*	NH_3-N*	NO_3^--N*
Average	8.37	1.56	156.07	75.08	28.20	5.99
Std. dev.	0.19	0.43	41.02	23.59	12.84	5.54
Min	7.89	0.90	78.40	39.20	11.03	1.00
Max	8.65	2.80	235.20	140.00	53.78	18.82

*Values in mg/l

(b) Microbial characteristics of secondary treated wastewater used to feed as influent for four constructed wetland units (Values in log MPN/100ml)

Parameter	***TC***#**(n=42)**	***FS*****(n=42)**	***S.typhi*****(n=21)**
Average	5.49	4.87	2.73
Std. dev.	0.58	0.53	0.29
Min	4.11	3.70	2.30
Max	6.20	5.95	3.48

Concentration of all the parameters in influent varied considerably throughout the monitoring period and is evident from the plots of pollutant parameters in Figure 4.1. High variations are found in the concentration of organic matter as well as nutrients. The microbial characteristics, which is the focus of the present study, shows that secondary treated wastewater was associated with significant levels of contamination of coliform and pathogens. In the research study microbial contamination has been evaluated in terms of two indicator species - *Total coliform* and *Fecal streptococci*, and one pathogenic specie - *Salmonella typhi*. The one standard deviation interval around the mean number of *Total coliform*, *Fecal streptococci* and *Salmonella typhi* were observed to be 5.49±0.58, 4.87±0.53 and 2.73±0.29 log MPN/100ml, respectively. These observed values are close to the reported numbers of indicator organisms in effluents of other STPs in India (Jamwal and Mittal, 2009). Also, the observed results are in accordance with the observations stated in the reports of national level government bodies such as NRCD, and CPCB in India.

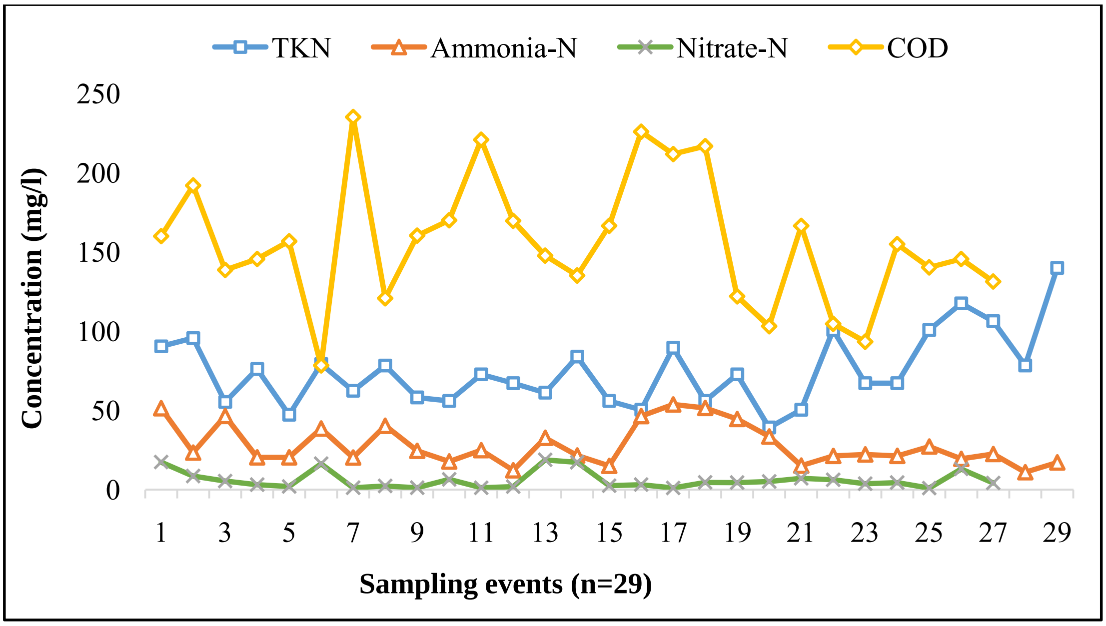

Fig. 4.1. Variations observed in values of secondary treated wastewater for Total Kjeldahl nitrogen (TKN), ammonia-nitrogen, nitrate-nitrogen and COD, throughout the study period

From the above results it is clear that the sewage treatment plants in India are mainly designed for organic matter and nutrient removal only, whereas the removal of microbial contaminants has been ignored. The existing treatment plants serve primarily to meet the standards for biological oxygen demand (BOD) and suspended solids, while 2-3 log reduction of coliform is incidental and does not meet the prescribed standards. The observed values during experimental analysis and in reported literature shows that the number of *Total coliform* are exceeding the standard limits of 1000MPN/100ml as suggested by WHO (1989) for safe disposal. Hence, it is clearly established that the high health risks associated with the effluents from sewage treatment plants (STPs) established in India need to be addressed. Thus, it indicates that there is a need for tertiary treatment step at STPs, wherever it is absent, to achieve the recommended standards for coliform reduction as suggested by NRCD (National River Conservation Directorate); 1000MPN/100ml Fecal coliform is desirable and 10,000MPN/ml is the maximum permissible limit for discharge into water bodies.

Table 4.3 gives the comparative insights of observed mean values of contaminants present in the secondary treated wastewater along with the prescribed Indian standards for their corresponding values. The comparison provides meaningful information to decide the suitable fate of the treated wastewater.

Table 4.3

Comparison of observed values of contaminants in the secondary treated wastewater with Indian standards

S. No.	Parameter	Observed mean concentration	Standard by CPCB for land irrigation	Standard by CPCB for disposal into water stream
1.	**pH**	8.37	5.5 to 9.0	5.5 to 9.0
2.	**COD***	156.07	250	-
3.	**TKN***	75.08	-	100
4.	**NH_3-N***	28.20	50	-
5.	**NO_3^--N***	5.99	-	10
6.	**Total Coliform (log_{10}/100ml)**	5.49	-	-

*Value in mg/l

* No standards for *Total coliform*: WHO (1989) prescribes 1000MPN/100ml

COD concentration in the treated effluent was observed to be in the range of 78.42mg/l to 235.20mg/l with mean concentration of 156.07± 41.02 mg/l. High variation for COD values could be related to the influent loading of raw domestic wastewater at the sewage treatment plant. The mean COD concentration was within the discharge standard limit of 250mg/l as given in Table 4.3. It indicates that the STP's effluent ranges within the prescribed standard limits suitable for discharge into water streams and land irrigation. It reflects the good performance efficiency of the treatment plant in terms of COD and that the maximum value could have been resulted from failure of treatment process due to some technical faults at STP site.

During the study period the concentrations for Total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH_3-N) and nitrate nitrogen (NO_3^--N) were observed to be in the range of 39.20-140mg/l, 11.03-53.78mg/l and 1-18.82mg/l, respectively with mean concentrations of 75.08±23.59mg/l, 28.20±12.84mg/l and 5.99±5.54mg/l, respectively, suitable for effluent disposal into water streams. These parameters concentrations, of secondary treated effluents sourced from a STP, are comparable to the corresponding values reported by Ghosh and Gopal (2010) for treated effluent sourced from milk processing plant. It can be seen that there is a significant variation in these nutrient parameters which could be due to the variation in strength of domestic sewage or could be related to the plant's performance subjected to technical/power failures.

From the above results it is clear that the secondary treated wastewater contains low concentrations of nutrients and organic matter, but possesses high concentrations of microbial contaminants. Thus, the purpose of further treatment is not nutrient or organic matter removal, but it is reduction of microbial contaminants to reduce the impact of health risks associated with the treated effluent. Now, in the order to produce the high quality effluent, particularly in regions where large land availability is a constraint, vertical flow constructed wetland is found to be a suitable and efficient eco-friendly system to achieve the standard limits for the microbiological parameters. The vertical flow constructed wetland system selected on the basis of literature review needs to be further examined on the basis of experimental results. The following section studies the performance of four constructed wetlands through analysis of physico-chemical and microbiological parameters.

4.4 Performance Efficiency of Constructed Wetlands

The treatment efficiency of any system depends not only on the design parameters of the system components, the flow regime and the influent feed parameters, but biological and ecological conditions are also of major concern. The constructed wetland is a pool of many physical and biochemical processes that results into degradation of organic matter and utilization of nutrients. Removal processes of these contaminants are majorly governed by the microbial species present within the column of constructed wetland, which use the organic matter and nutrients for their growth and development; utilization of nutrients is also driven by plant species for their growth and development. Thus, these contaminants are the key parameters to study the efficiency of any wetland system.

In the present study, organic matter degradation is calculated in terms of chemical oxygen demand (COD) and reduction of nutrients is calculated in terms of removal of Total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH_3-N), nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N). The removal processes of the contaminants are also affected by the ecological conditions of the constructed wetland system; pH reflects the buffering conditions whereas dissolved oxygen (DO) content gives an estimation about the aerobic/anaerobic conditions prevailing in the system. Apart from all these, the unavoidable microbial contaminants are of major concern as they are related to health risks associated with them. The relative importance of removal of microbiological contaminants has already been discussed in the previous sections and this section will evaluate the efficiency of constructed wetlands in terms of both, physico-chemical parameters as well as microbiological parameters, with focus on the later.

4.4.1 Physico-chemical parameters

In order to monitor the sustainability of the treatment system, physico-chemical properties are taken into account, since reduction in organic matter content and nutrients are general basis for analysis of their performance. Such evaluation gives insights for biochemical processes carried out in the system and helps in evaluating the best possible environmental conditions prevailing in each system. In the proposed study, the physico-chemical analysis is performed as background analysis for all constructed wetland units while focus is on microbiological parameters.

4.4.1.1 Physical parameters (pH and DO)

The analysis of physical parameters of constructed wetland units reveal that there is no appreciable change in pH values from inlet mean value of 8.37 to outlet mean values of 8.27, 8.16, 8.37 and 8.28 for UN-ph, UN-cn, UN-ct and UN-cn-sd, respectively (Figure 4.2). This suggests that all the wetland units are well-buffered due to the presence of enough carbonate alkalinity in the system.

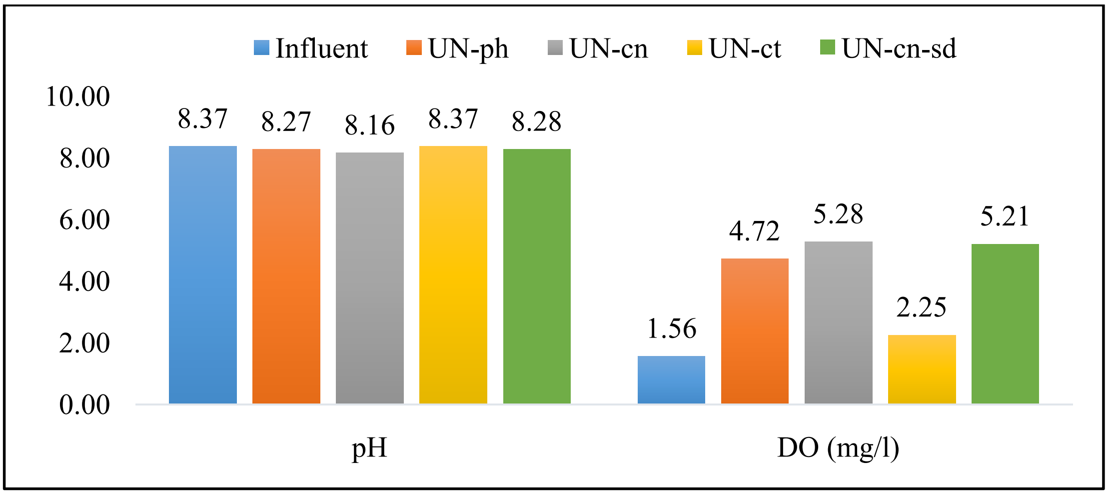

Fig. 4.2. Mean concentrations of physical parameters, pH and DO, in effluent of four constructed wetland units with corresponding mean influent concentration during the study period

Mean concentrations of dissolved oxygen (DO) was 1.56mg/l in influent for all units and it greatly increased to 4.72, 5.28, 2.25 and 5.21mg/l in the effluents of UN-ph, UN-cn, UN-ct and UN-cn-sd, respectively. The mean influent concentration of DO was far below the CPCB standard of 4mg/l or more for fisheries and wild life propagation. Because of the sewage disposal in water streams, concentration of DO starts depleting through use as COD. Below some threshold level, it threatens the aquatic life in the receiving water streams. Therefore, DO value has been assumed as good indicator of aquatic life in water bodies. In this view, constructed wetland systems in the proposed research, helped to increase the dissolved oxygen levels that can be sufficient to support the life in water streams for aquatic biota. Effluents of all three planted constructed wetland units showed significant increment in values of DO as compared to the control unit, which clearly signifies the presence of plants in constructed wetland units to increase the DO concentrations for safe disposal into water bodies.

4.4.1.2 Chemical parameters (COD, TKN, NH_3-N and NO_3^--N)

Chemical parameters include analysis of COD, TKN, NH_3-N and NO_3^--N in influent and effluent of all four constructed wetland units; COD measured for organic matter evaluation while TKN, NH_3-N, NO_2^--N and NO_3^--N measured as indicators of nitrogenous matter. Figure 4.3 represents the inlet and outlet concentrations of these parameters in planted units UN-ph, UN-cn and UN-cn-sd as well as unplanted unit UN-ct, operated at the same hydraulic retention time under vertical subsurface up-flow condition. Table 4.4 gives the results of statistical analysis performed on the experimental results for all four units and for all parameters. The concentration of NO_3^--N was measured but is not shown in the results as it was found to be below detectable level which showed the prevalence of aerobic conditions in all units.

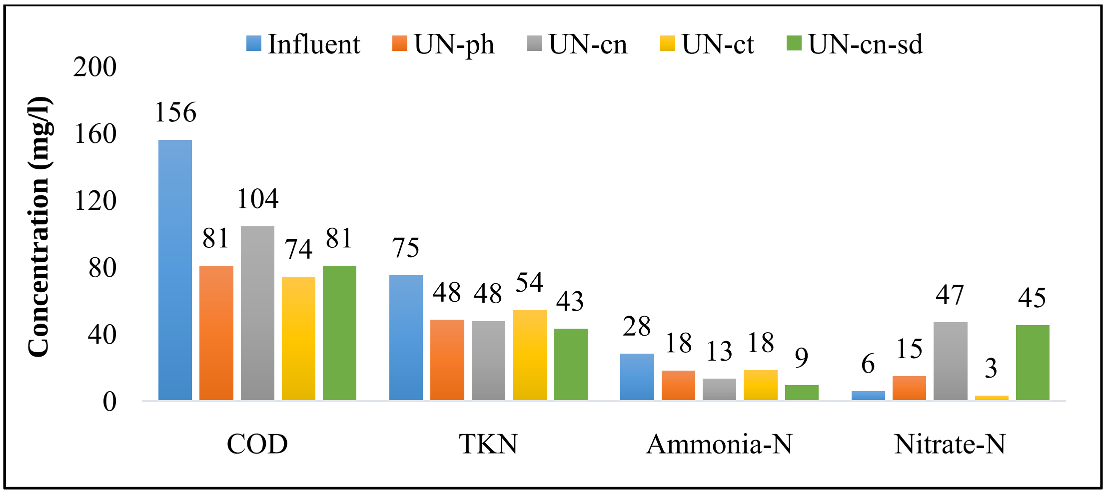

Fig. 4.3. Mean concentrations for chemical parameters, (COD, TKN, NH_3-N and NO_3^--N) in effluent of four constructed wetland units with corresponding mean influent concentration during the study period

Table 4.4

Difference in performances of different units estimated by statistical analysis using t-test at significance level of p-value<0.05 between removal efficiencies of various chemical parameters

Units / Parameters	TKN	NH_3-N	NO_3^--N	COD
UN-ph Vs UN-cn	N	S	S	S
UN-ph Vs UN-ct	N	N	S	N
UN-ph Vs UN-cn-sd	N	S	S	N
UN-cn Vs UN-ct	N	S	S	S
UN-cn Vs UN-cn-sd	N	N	N	S
UN-ct Vs UN-cn-sd	N	S	S	N

*S = significant difference, N= non-significant difference
For Nitrate-N the statistical analysis was done on outlet concentrations

Removal of COD**:** experimental results of the four constructed wetland units fed with secondary treated wastewater reveal that the mean percentage removal of COD was 47.6%, 34.6%, 54.6% and 51.2% from UN-ph, UN-cn, UN-ct and UN-cn-sd, respectively. Corresponding mean residual effluent concentrations are 80.9, 104.4, 74.2 and 80.8 mg/l for UN-ph, UN-cn, UN-ct and UN-cn-sd, respectively. The mean influent COD concentration of 156.1mg/l was found to be within the range of 109-193mg/l as reported by Gopal and Ghosh (2010). The 46 % removal of COD in the reported study was comparable with the observed 47.6% removal from UN-ph only. Whereas a study by Chang et al. (2012) found higher COD removal of 61.4% as compared to the observed removals of the proposed study, where the high percentage removal might be due to the high influent COD concentration of 288.68±27.4mg/l used in this reported study. The higher percentage of removal of COD from unplanted unit, UN-ct reflects similar observations as observed by Stefanakis and Tsihrintzis (2012).

Figure 4.4 shows the percentile curve of COD concentrations in effluent from four constructed wetlands corresponding to their influent concentration along with the standard values for COD (250mg/l). It can be observed that none of the percentile curves crosses the standard value line. Hence, comparison of the percentile distributions reveals that all the treatment systems were capable of robustly treating secondary treated wastewater. It also shows that the COD concentration of influent was already within the limits i.e. below 250mg/l according to the Indian standards for discharge into water bodies. And reduction of COD was achieved in all the treatment units, with highest percentage removal observed for UN-ct; the trend line for UN-ct shows that the 80% effluent distribution is found to be below 102.1 mg/l.

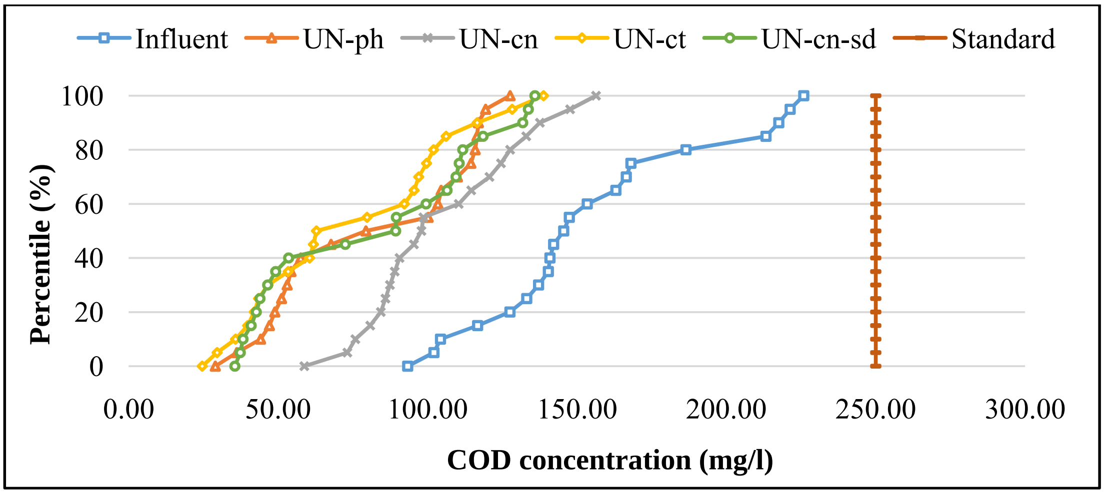

Fig. 4.4. Percentile curve for all four constructed wetland units removing organics, COD, from secondary treated wastewater during the study period

Statistical analysis was performed to analyze the performance of different wetland units. All the units were found to be capable of robustly treating secondary treated wastewater as was evident from the significant difference (p-value<0.05) between influent and effluent concentrations of constructed wetland units. Effluent concentration of UN-cn was found to be significantly different, with low concentration, as compared to rest of the three units. While no significant difference was observed among the effluent concentrations of rest of the three units for COD (Table 4.4).

***Nitrogenous contaminants*:** Nitrogenous contaminants were measured in terms of TKN, NH_3-N, and NO_3^--N (concentration of NO_2^--N, as reported earlier, was found to be below detectable level and hence was not considered for further analysis). Such contaminants are removed due to the treatment functionality of any system through processes such as plant uptake, nitrification, denitrification and other biochemical processes.

Total Kjeldahl nitrogen (TKN) is measured as a sum of organic nitrogen and ammonia-N. The mean concentration of TKN in influent was found to be 75.1mg/l during the study period. The mean percentage removal were 37.8%, 39.7%, 34.9%

and 44.1% corresponding to the mean effluent concentrations of 48.5, 47.7, 54.3 and 43.1mg/l from UN-ph, UN-cn, UN-ct and UN-cn-sd, respectively. Figure 4.5 shows the percentile curve of TKN concentrations in effluent from four constructed wetlands corresponding to their influent concentration along with the standard values for TKN (100mg/l). It can be seen that 85% of total observations of influent concentrations were within the standard limits. As a treatment function, when the effluent concentrations were statistically compared with influent concentrations, all four units were found to be significantly efficient (p-value<0.05) in removing TKN. At the same time when comparison was made among the effluent concentrations of different units, no significant difference was found (Table 4.4). The lowest percentage removal of TKN from the control unit UN-ct as compared to the planted units UN-ph, UN-cn and UN-cn-sd, supports the significance of presence of plants in constructed wetlands for the removal of TKN. Comparatively, high average percentage removal of TKN was achieved by UN-cn-sd followed by UN-cn.

The mean concentration of ammonia-N in influent was found to be 28.2mg/l during the study period. The mean percentage removal were 35.5%, 51.0%, 34.4% and 65.2% corresponding to the mean effluent concentrations of 18.2, 13.2, 18.4 and 9.2mg/l from UN-ph, UN-cn, UN-ct and UN-cn-sd, respectively. Percentile curve shown in Figure 4.6 shows that all the treatment units were capable for conversion of ammonia-N. The influent concentration distribution reflects that 90% of concentrations remain within the standard limits while the only 10% exceeds the standard limit during the study period. Percentile curves for UN-cn-sd shows that the ammonia-N concentration never exceeded 21.09mg/l in effluent, irrespective of the influent concentration. All the treatment units were found to be efficient for conversion of ammonia-N. Significant difference was observed in effluent concentrations of constructed wetland units when compared with each other (p-value<0.05), except the difference between UN-ph and UN-ct and the difference between UN-cn and UN-cn-sd (Table 4.4).

The mean concentrations of NO_3^--N were found to increase from 6.0mg/l in influent to 14.8, 47.0, and 45.1mg/l in the effluents of UN-ph, UN-cn and UN-cn-sd respectively, while effluent concentration decreased to 3.1mg/l in effluent from UN-ct. The significant increment of nitrate-N concentration in UN-cn and UN-cn-sd clearly indicates that the presence of aerobic conditions dominated in these two units that enabled the nitrification process to increase the concentration. The increased nitrate-N concentrations in effluents of all the planted units was in accordance with the study by Stefanakis and Tsihrintzis (2012). Statistical analysis shows that there was a significant difference between influent and effluent concentrations of all constructed wetland units. All the effluent concentrations were significantly different when compared among the treatment units except between UN-cn and UN-cn-sd where no significant difference in effluent concentrations were observed (Table 4.4). It seems that incorporation of sand layer does not contribute for any significant change in the concentration of NO_3^--N.

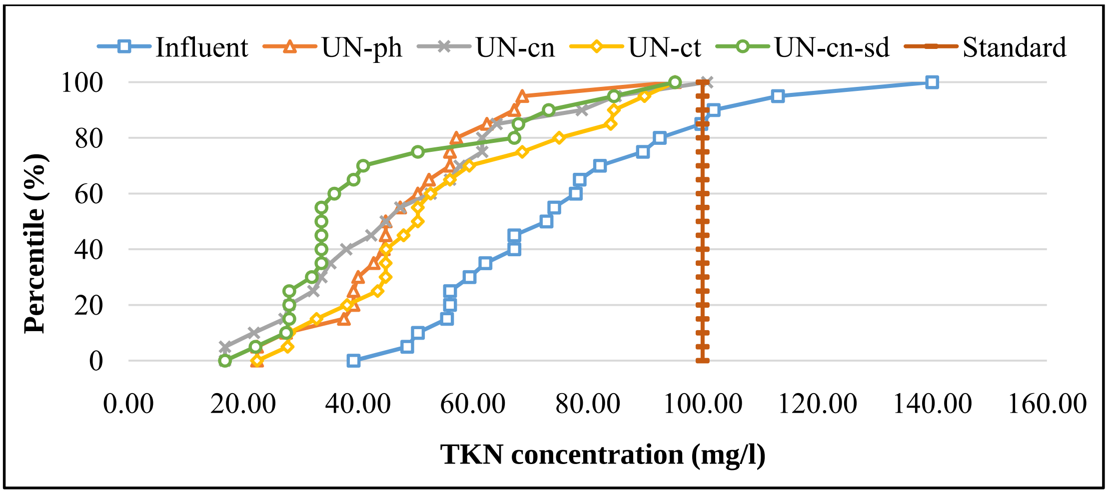

Fig. 4.5. Percentile curve for all four constructed wetland units removing TKN from secondary treated wastewater during the study period.

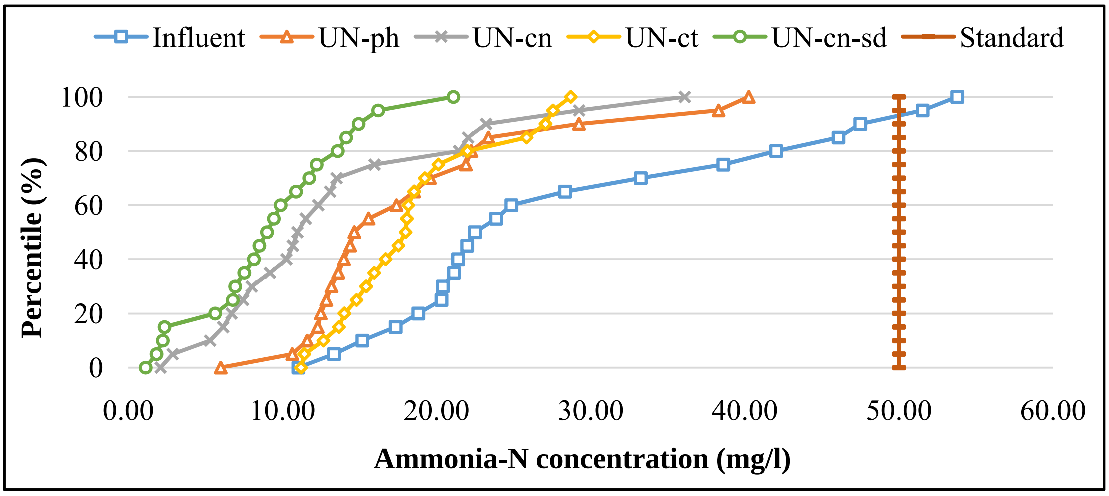

Fig. 4.6. Percentile curve for all four constructed wetland units removing ammonia-N from secondary treated wastewater during the study period

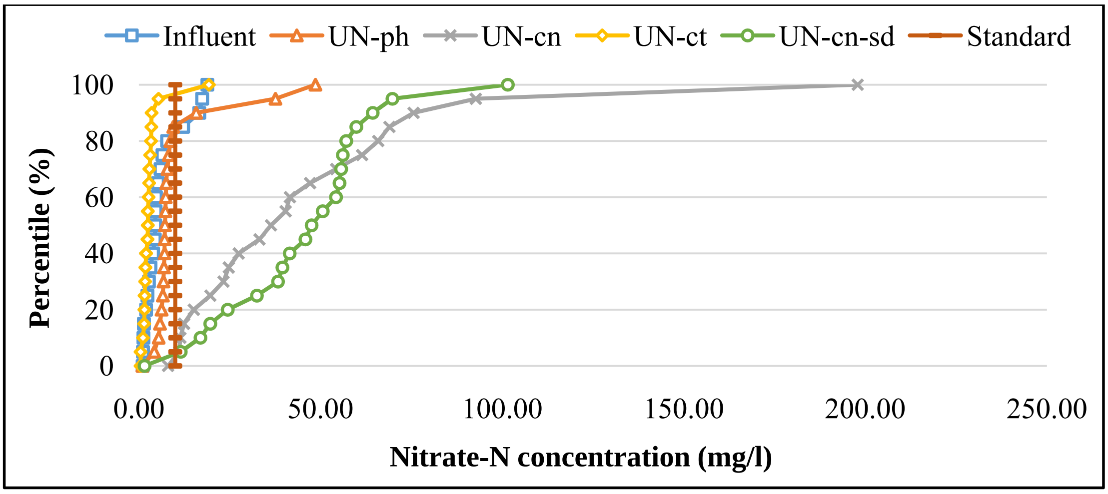

Fig. 4.7. Percentile curve for all four constructed wetland units removing nitrate-N from secondary treated wastewater during the study period

4.4.1.3 Possible environmental conditions prevailing with in each wetland system

The physico-chemical analysis was done in order to identify the functionality of each treatment unit which ultimately suggests the possible environmental conditions prevailing in each treatment wetland unit. Table 4.5 presents the comparative account of mean effluent characteristics of all constructed wetland units proposed in the study with their mean influent concentrations and respective percentage removal efficiencies.

Table 4.5

Comparative insight of mean influent and effluent concentration in mg/l with their corresponding removal efficiency for all four treatment units (n=20)

Parameters	Influent	UN-ph	Removal	UN-cn	Removal	UN-ct	Removal	UN-cn-sd	Removal
COD	156.1	80.9	47.6%	104.4	34.6%	74.2	54.6%	80.8	51.2%
TKN	75.1	48.5	37.8%	47.7	39.7%	54.3	34.9%	43.1	44.1%
NH_3-N	28.2	18.2	35.5%	13.2	51.0%	18.4	34.4%	9.2	65.2%
NO_3^--N	6.0	14.8	-	47.0	-	3.1	-	45.1	-
pH	8.37	8.27	-	8.16	-	8.37	-	8.28	-
DO	1.56	4.72	-	5.28	-	2.25	-	5.21	-

The results signify the presence of plant in removal of nutrient contents as evident by relatively higher percentage removal of TKN and NH_3-N from planted units (UN-ph, UN-cn and UN-cn-sd) as compared to the unplanted unit (UN-ct). Comparison among planted units shows that UN-cn and UN-cn-sd are superior for removal of nutrient contaminants. These observations support the significance of presence of *Canna* *indica* for constructed wetland treating secondary treated wastewater. Higher mean percentage removal of nutrients in terms of TKN and ammonia-N from *Canna indica* planted wetlands were coherent with the study by Chang et al. (2010), where the author found the *Canna spp*. as a competitive plant species for nutrient removal. Also, the percentile curve for removal of ammonia-N from UN-cn-sd already showed that the ammonia-N concentration never exceeded 21.09mg/l in effluent, irrespective of the influent concentration (Figure 4.6), which supports the higher performance efficiency of UN-cn-sd. Moreover, the performance efficiency for NH_3-N removal was in order UN-cn-sd>UN-cn>UN-ph>UN-ct.

The concentration of nitrate-N significantly increased in effluent of all the planted units (UN-ph, UN-cn and UN-cn-sd) and this increased concentration of NO_3^--N with simultaneous removal of ammonical-N was a result of nitrification process in highly aerobic conditions. Whereas, the removal of nitrate-N was observed in unplanted UN-ct units and the reason may be attributed to heterotrophic denitrification in anoxic conditions in absence of plants. Even the higher concentration of dissolved oxygen (DO) indicates the possibility of higher nitrification process in UN-cn-sd that could have caused the high concentration of nitrates through removal of ammonia-N. When comparing the planted units, UN-cn and UN-ph, it can be seen that the concentration of nitrate-N was higher in *Canna* planted unit as compared to the *Phragmites* planted unit. The results from planted the unit UN-ph, and the unplanted unit UN-ct, does not greatly differ for the removal of nutrients (Table 4.5). This observation is in agreement with the study by Torrens et al. (2009) which did not found significant, the presence of *Phragmites australis* for removal of nitrogenous species. The above observation does not imply that the *Phragmites* spp. is unsuitable for nutrient removal from wetland units, in fact they signify that the *Canna* spp. is a better choice and its presence enhances the favorable conditions for removal of nutrients.

***Removal mechanism*:** The experimental analysis shows that the nutrient removal might be resulted from the significant capacity of vertical flow regime that enables the nitrification process and plant uptake through effective wastewater-root zone contact (Moreno et al., 2002). The nutrient removal due to the nitrification process could reflect the presence of aerobicity within the system. The experimental results for nutrient removal confirms the study that the vertical flow constructed wetlands possess high oxidation capacity due to high oxygen transfer (Brix and Arias 2005; Kadlec and Wallace 2009). At the same time, the high percentage removal also shows the possibility of high rate of biochemical processes.

Removal of organic matter (COD) is majorly driven by physical processes (Li et al., 2013) as well as microbial processes both. The former separates the organics allowing their hydrolysis for degradation and later includes the microbial growth that helps to catalyze chemical reactions including the utilization of oxygen present within the system (IWA, 2000). Although the analysis of COD results elucidates that the presence of plant or use of different plant species does not greatly affect the removal of organic matter, however, organic matter removal could have been possibly occurred due to the cumulative effect of different biochemical and physical processes (filtration, sedimentation, adsorption, etc.). The higher percentage removal of COD from UN-cn-sd as compared to UN-ph and UN-cn reveals that the physical processes might be dominating the situation. But the statistical analysis result, which shows that there is no significant difference between effluent of UN-cn-sd and UN-ct (Table 4.4), shows that the impact of ecological conditions prevailing in the system could not be avoided. The highest removal of COD in the unplanted unit UN-ct can be correlated with the removal of other parameters. The most likely processes for nutrient contents removal are nitrification/denitrification processes and plant uptake. All such mechanisms occur simultaneously within the system. When wastewater is first applied to the system, heterotrophic organism starts utilizing the oxygen and degrades the organic compounds for organic matter mineralization, where the supply of oxygen is an important factor (Truu et al., 2009). Simultaneously, nitrifiers utilize the oxygen for nitrification and produce NO_3^--N at the same time. If competition occurs between heterotrophic organisms and nitrifiers (Truu et al., 2005), anoxic zone might be created which subsequently causes the heterotrophic denitrification. These conditions could have happened in UN-ct, as in the absence of plant species not enough oxygen was transported; the results show that the DO value at the mid-point of UN-ct was 1.01mg/l. As a result, denitrification could have reduced oxidized forms of nitrogen in response to the oxidation of an electron donor such as organic matter. As denitrifying microbes require very low oxygen concentration (<10%) and require organic carbon for energy, additionally contributing towards higher removal of COD from the unplanted unit UN-ct.

It is now clear that the observed removal of nitrate-N and the maximum mean percentage removal of COD from the unplanted UN-ct unit might be due to the heterotrophic denitrification in anoxic conditions in absence of plants. Additionally the reduction of NH_3-N in this unit might be possible through a process called anaerobic ammonium oxidation (ANAMMOX) in anoxic conditions. Thus, it can be clearly shown that the simultaneous removal of NH_3-N and NO_3^--N in UN-ct is resulted from the design feature of up-flow regime where oxic conditions prevailed in the upper zone (i.e. below the surface layer) and slightly anoxic conditions might be present in the bottom zone. It shows the possible simultaneous occurrence of nitrification and heterotrophic denitrification processes (including ANAMMOX) in the control unit. Possibility of loss of NH_3-N by volatilization is negligible in all the units as it is not significant below the 9.5 pH level (IWA 2000; Poach et al., 2004), which is in accordance with the observed pH values.

The above discussion and analysis of physico-chemical parameters suggest that the removal of nutrients was greatly affected by the presence of plant and plant species. *Canna indica* was found to be the best suitable plant species for nutrients removal and removal can be enhanced by creating gradation effect through inclusion of finer material such as sand (UN-cn-sd) that enables highly porous system. These analyses give the overall insights for all four well established constructed wetlands which reveal that UN-cn-sd unit, being the highly aerated, is overall the best suitable unit for secondary treated wastewater in terms of removal of physico-chemical parameters. The environmental consideration of the treated effluent from this unit allows for its safe disposal into water streams in terms of physico-chemical parameters. Although this was not the objective of the study it has helped to achieve the best “Compact-Eco-Technology” for the tertiary treatment.

4.4.2 Microbiological parameters (*Total coliform, Fecal streptococci* and S*almonella typhi*)

The study of microbiological parameters is the focus of proposed study as characteristics of secondary treated effluent clearly indicated the presence of coliform in an amount which is enough to pose public health risks. With such purpose of the study, secondary treated wastewater was applied to all constructed wetland units. Three species, two indicators species *Total coliform* and *Fecal streptococci* and one pathogenic specie *Salmonella typhi* were detected at inlet as well as outlet of the constructed wetland units.

Table 4.6
Comparative insight of mean influent and effluent concentrations (log MPN/100ml) for the three microbial species from all four treatment units

Constructed wetlands	*Total coliform*	*Fecal streptococci*	*Salmonella typhi*
Influent concentration	**5.49**	**4.87**	**2.73**
UN-ph	4.30	3.60	1.51
UN-cn	3.88	3.42	1.69
UN-ct	4.31	3.74	0.50
UN-cn-sd	2.75	2.44	0.83

The performance efficiency of removal of microbial contaminants from the system was evaluated in terms of log removal with focus on minimizing the mean effluent concentration (Table 4.6). Furthermore, based on the observed results, UN-cn-sd was focused and the presence of the finer material sand was evaluated extensively.

The observed results of the proposed study are first presented and then compared with the previously reported theories while confirming the efficiency of the treatment systems. Further, the removal patterns are correlated with other parameters to infer about the mechanism followed and factors affecting the removal of microbial species.

4.4.2.1 Experimental and statistical analysis

The concentrations of microbial species in the influent and effluents of the various treatment units were analyzed and the results are presented in the Figures 4.8, 4.9, 4.10. Mean influent concentrations of *Total coliform, Fecal streptococci* and *Salmonella typhi* were observed to be 5.49, 4.87 and 2.73 log MPN/100ml, respectively. From the mean effluent concentrations, the mean log removal was calculated for each of the microbial species within each constructed wetland as given in Table 4.7. The mean log removal from UN-ph is 1.01, 1.01 and 1.20 log units, from UN-cn is 1.87, 1.82 and 1.09 log units, and from UN-ct is 1.24, 1.03 and 2.02 log units for *Total coliform, Fecal streptococci* and *Salmonella typhi,* respectively. As discussed earlier, on the basis of higher performance efficiency of UN-cn, UN-cn-sd was designed as an improved system. Table 4.6 shows that the minimum mean outlet concentrations of indicator species (*Total coliform* and *Fecal streptococci*) are observed in the effluent from UN-cn-sd and the mean effluent concentration of *Salmonella* specie is also considerably low from UN-cn-sd. The log removal from the UN-cn-sd unit is found to be 3.01, 2.80 and 1.58 log units for *Total coliform, Fecal streptococci* and *Salmonella typhi,* respectively.

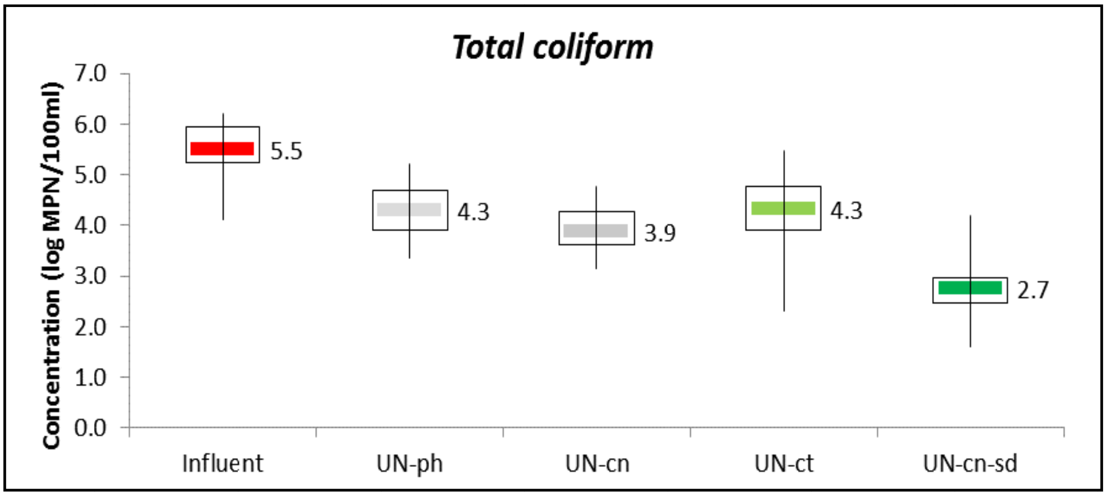

Fig. 4.8. Comparative insight of mean influent and effluent concentrations (log MPN/100ml) for the *Total coliform* from all four treatment units

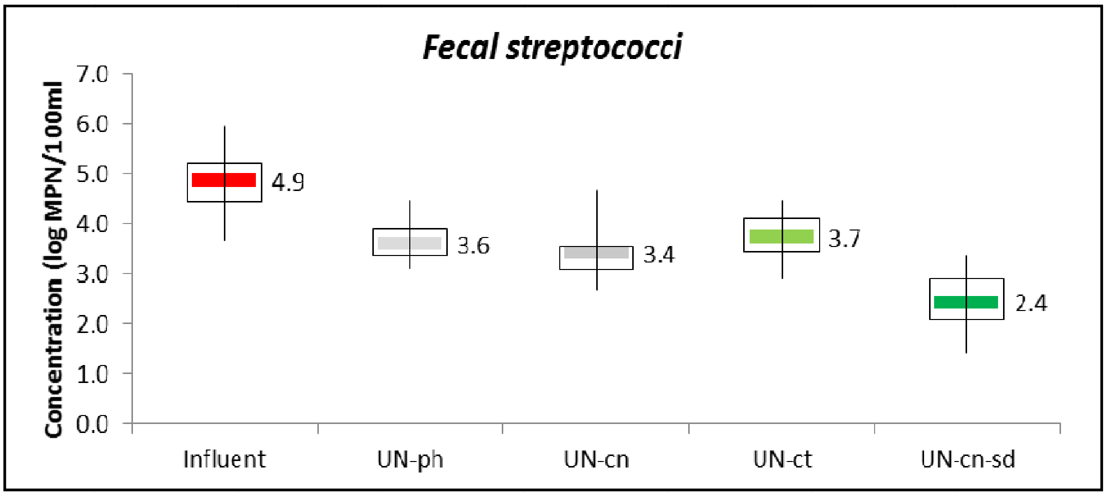

Fig. 4.9. Comparative insight of mean influent and effluent concentrations (log MPN/100ml) for the *Fecal streptococci* from all four treatment units

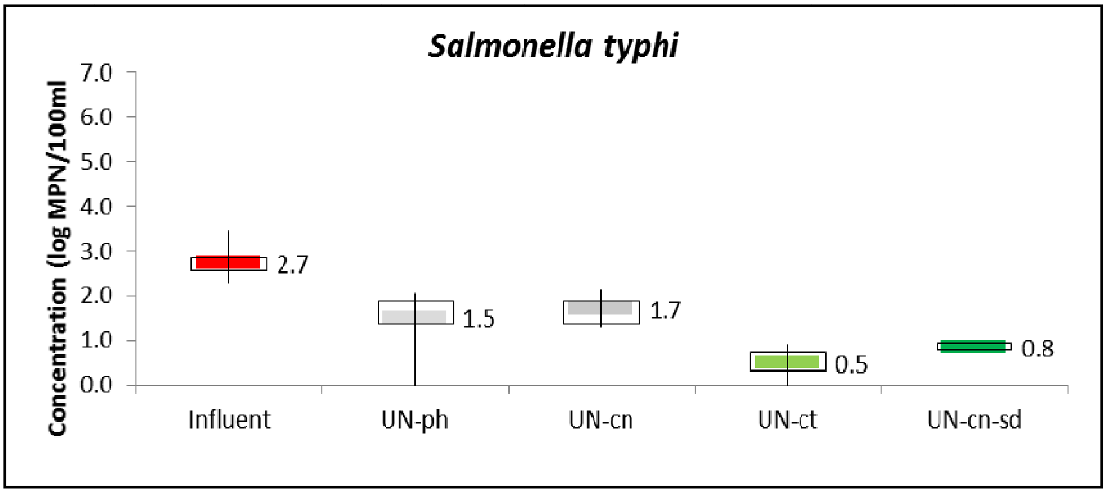

Fig. 4.10. Comparative insight of mean influent and effluent concentrations (log MPN/100ml) for the *Salmonella typhi* from all four treatment units

Table 4.7

Mean log removal for three microbial species observed within four constructed wetland units

Units	*Total coliform*	*Fecal streptococci*	*Salmonella typhi*
UN-ph	1.01	1.01	1.20
UN-cn	**1.87**	1.82	1.09
UN-ct	1.24	1.03	**2.02**
UN-cn-sd	**3.01**	2.80	1.58

These results reveal that the highest log removal of indicator organisms *Total coliform* and *Fecal streptococci* has been observed for the unit UN-cn-sd followed by UN-cn, UN-ct and UN-ph, in decreasing order, while pathogenic specie *Salmonella typhi* was best removed by the unit UN-ct followed by UN-cn-sd, UN-ph and UN-cn, in decreasing order. These results clearly indicate that UN-cn-sd greatly improves the efficiency of removal of indicator organisms and also improves the removal of *Salmonella typhi*. The results also indicate different removal mechanisms for indicators and pathogenic species in constructed wetland unit.

The above results are further analyzed in detail with the help of percentile curves. Percentile curves represent the distribution of effluent concentrations with the corresponding distribution of influent concentrations. These curves give a comparative information of distribution of effluent concentrations throughout the monitoring period among the different units for each of the microbial species. These curves clearly indicate that all the treatment units are significantly efficient in removal of the aimed three species from the secondary treated wastewater.

Figure 4.11 shows percentile curves of all the units for the effluent concentrations (in log scale) of *Total coliform*. The curves clearly show that UN-cn is more efficient than UN-ph and UN-ct, wherein the curves of UN-ph and UN-ct was close to each other. More importantly, the curve for UN-cn-sd stood separately and shows that 90% of the observed effluent concentrations falls within the WHO recommended standard of 1000MPN/100ml (3logMPN/100ml). The mean *Total coliform* concentration in the effluent from UN-cn-sd was found to be 2.75logMPN/100ml whereas the 90th percentile concentration value was found to be 3.03logMPN/100ml. This curve confirms the high removal efficiency of coliform numbers from the secondary treated wastewater for the unit UN-cn-sd as was observed from the mean effluent concentrations.

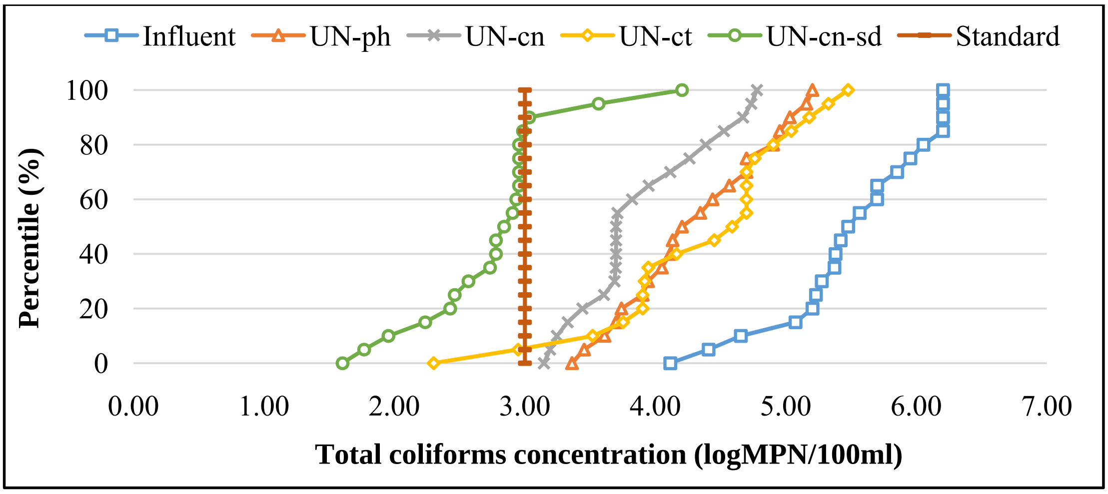

Fig. 4.11. Percentile curves for *Total coliform* concentration (log MPN/100ml) in influent and effluents from four constructed wetland units

Figure 4.12 presents the distribution of effluent concentrations of *Fecal streptococci* along with the corresponding influent concentration distribution observed throughout the study period. Percentile curves for *Fecal streptococci* reveal that all the treatment units were capable of reducing the levels of *Fecal streptococci* with the unit UN-cn-sd being the most robust unit with 90% of the observed concentrations falling below the concentration of 2.90logMPN/100ml. The removal of *Fecal streptococci* from rest of the three units (UN-ph, UN-cn and UN-ct) follows the same pattern as shown for *Total coliform.* But, the curves for *Fecal streptococci* were sturdier (less varying) than the curves observed for *Total coliform* which shows that *Fecal streptococci* is more resistant to the changing environmental conditions prevailing within the system.

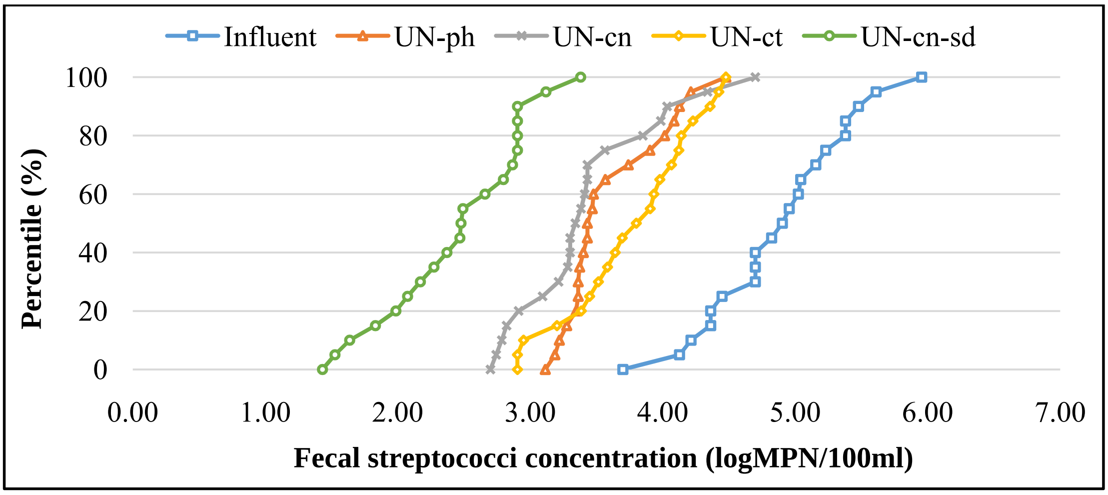

Fig. 4.12. Percentile curves for *Fecal streptococci* concentration (log MPN/100ml) in influent and effluents from four constructed wetland units

The inferences drawn above for both, *Total coliform* as well as *Fecal streptococci*, are also supported by the statistical analysis of the experimental results, where significant difference was found between removal from the units UN-ph and UN-cn but the difference between removal from UN-ph and UN-ct were found to be statistically non-significant (Table 4.8). These results indicates that the presence of *Phragmites australis* have negligible impact whereas the presence of *Canna indica* could be of importance for deciding the fate of indicator organisms. Furthermore, the significant difference observed between the removal from UN-cn-sd as compared to UN-ph, UN-cn and UN-ct, again confirms the result of highest efficiency from UN-cn-sd for these two indicator species, *Total coliform* and *Fecal streptococci*.

Table 4.8

Difference in performances of different units estimated by statistical analysis using t-test at significance level of p-value<0.05 between removal efficiencies of various microbial contaminants

Units / **Parameters**	**TC**	**FS**	***S.typhi***
UN-ph vs UN-cn	S	S	N
UN-ph vs UN-ct	N	N	S
UN-ph vs UN-cn-sd	S	S	S
UN-cn vs UN-ct	S	S	S
UN-cn vs UN-cn-sd	S	S	S
UN-ct vs UN-cn-sd	S	S	N

*S = significant difference, N= non-significant difference

Figure 4.13 presents the distribution of effluent concentrations of *Salmonella typhi* along with the corresponding influent concentration distribution observed throughout the study period. Percentile curves with different trend for *Salmonella typhi* elucidated that although all the treatment units are efficient to reduce the level of *Salmonella typhi* from the secondary treated wastewater, the highest mean removal efficiency for this pathogen was observed in the unit UN-ct followed by UN-cn-sd, UN-ph and UN-cn, in decreasing order. The figure shows that the curve for UN-ct was closer to the UN-cn-sd curve, as compared to UN-ph and UN-cn, which clearly confirms that these two units, UN-ct and UN-cn-sd, were more capable for removing *Salmonella typhi* than UN-cn and UN-ph. Such trends are also in agreement with the statistical analysis where the removal of UN-ct and UN-cn-sd was found to be non-significant, as shown in Table 4.8. Also the statistical results shows that there is no significant difference between removal from UN-cn and UN-ph, which confirms that the effect of using plant species is negligible on removal of *Salmonella typhi*.

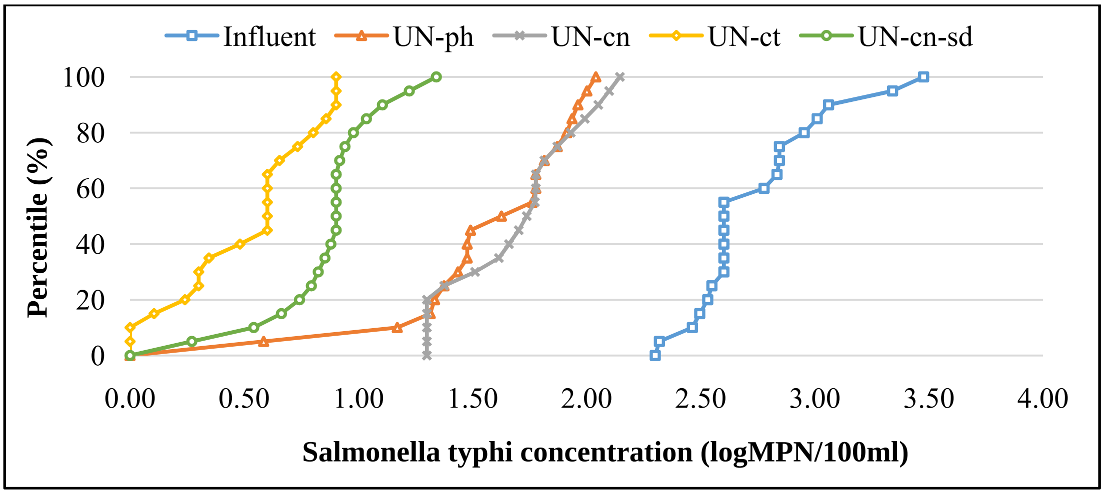

Fig. 4.13. Percentile curves for *Salmonella typhi* concentration (log MPN/100ml) in influent and effluents from four constructed wetland units

4.4.2.2 Analysis of UN-cn-sd for indicator organisms: middle point analysis

UN-cn-sd is found to be the most effective unit in terms of both physico-chemical and microbial parameters. In order to justify its significance, each aspect related to its development and design demands an independent analysis. Keeping this objective in mind, in order to know the effect of finer material sand as well as to understand the microbial removal efficiency in different zones, the Un-cn-sd unit has been studied via middle point sampling too, along with sampling at inlet and outlet points. The transverse cross-section of the wetland unit UN-cn-sd shows that below the middle point, a layer of smaller sized (8-12mm) gravels was placed above the larger sized (16-20mm) gravels, such that the smaller sized gravels accounts for only 30% of the total area from inlet to middle point. Whereas above the middle point smaller sized (8-12mm) gravels and finer material sand (1-2mm) occupy the total area. The middle point analysis has only been performed for indicator microorganisms (*Total coliform* and *Fecal streptococci*).

Figure 4.14 and 4.15 present how the concentration of *Total coliform* and *Fecal streptococci* varies during the monitoring period at inlet, middle and outlet points of the wetland unit. The observed mean concentrations for *Total coliform* were 5.36, 4.89 and 2.72 log MPN/100ml and for *Fecal streptococci* are 4.40, 4.13 and 2.00 log MPN/100ml at inlet, middle and outlet points of the unit, respectively. Mean log removals for *Total coliform* were 0.48 and 2.64 and for *Fecal streptococci* were 0.27 and 2.40 from middle and end points, respectively. Significantly lower outlet concentrations were observed from the outlet point as compared to the middle point. The concentration distributions reveal that the incorporation of finer material sand greatly improves the performance of the wetland unit.

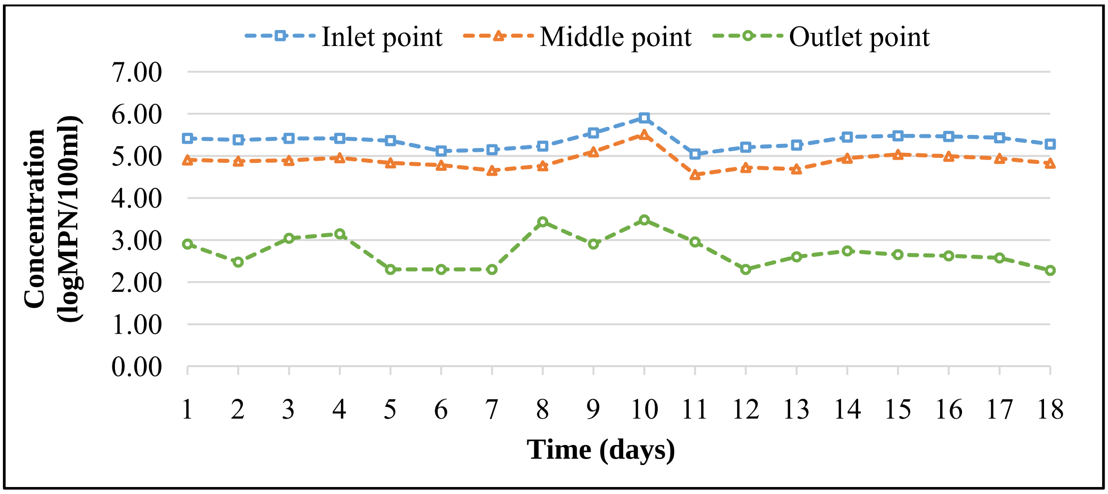

Fig. 4.14. Concentration of *Total coliform* (log MPN/100ml) at inlet, middle and outlet points of UN-cn-sd during the monitoring period

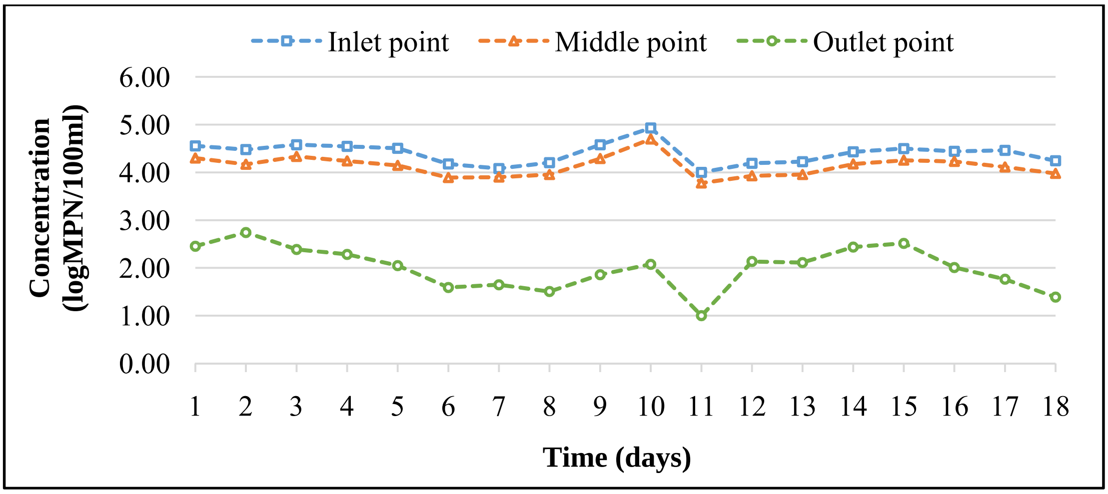

Fig. 4.15. Concentration of *Fecal streptococci* (log MPN/100ml) at inlet, middle and outlet points of UN-cn-sd during the monitoring period

4.4.2.3 Results comparison with reported studies

The proposed constructed wetland units were found to be efficient for removal of microbial contaminants. The removal of *Total coliform* from the *Canna* planted units, UN-cn (1.87 log units) and UN-cn-sd (3.01 log units), were found to be comparatively higher than the removal of 1.52 log units observed in the study by Gersberg et al. (1989b) which used *Scirpus* planted wetland for treating secondary treated effluent. The removal of indictor organisms, *Total coliform* and *Fecal streptococci*, from the *Canna* planted units were also found to be higher than the study by Arias et al. (2003), where the reduction was observed in the range of 0.7-1.5 log unit.

The study by Garcia et al. (2003) found that the removal of microbial indicators (Fecal coliform and *Somatic coliphages*) were higher when treating secondary treated wastewater through horizontal constructed wetland with finer media as compared to the similar wetland with coarser granular media. These comparisons support the higher efficiency for removal of microbial contaminants from the unit UN-cn-sd containing finer material (sand) as compared to the other units with coarser media. The mean removal of *Total coliform* from the unit UN-cn-sd (3.01 log units) lies in the range of 2.7-4.7 log units as observed in the study by Kadam et al. (2008), where domestic wastewater was treated through vertical flow constructed wetland. Whereas the removal of *Fecal streptococci* from the same unit UN-cn-sd (2.80 log units) was found to be higher than the range suggested by the study (1.7-2.5 log units).

4.4.2.4 Factors affecting the removal of microbial species

Among the initially developed three units, UN-ph, UN-cn and UN-ct, the best performance was shown by UN-cn for removal of nutrients as well as microbial indicators. The unit has same characteristics as of UN-ph and UN-ct in terms of media type and distribution, except the difference in plantation; unit UN-cn is planted with *Canna indica*, UN-ph planted with *Phragmites australis* and UN-ct is the unplanted control unit. The high removal from UN-cn could be attributed to the presence of *Canna indica* plant. Presence of *Canna indica* has already been proved significant for removal of nutrients from wastewater in various studies (Zhang et al. 2007; Konnerup et al. 2009). However, its suitability in terms of microbial contaminant removal in different environmental conditions needs to be further explored, as there is only limited knowledge shared through few reported studies (Zurita et al. 2006; Chang et al. 2010).

The high performance of *Canna indica* planted unit UN-cn has been related to its higher capacity of phytoremediation and to its fibrous rooting system with capability of high root zone aeration, larger root surface area and high root numbers (Wenyin et al. 2007; Zhang et al. 2007; Bose et al. 2008). The increased root surface area helps to enhance the physical mechanisms such as aggregation, adsorption, oxidation, sedimentation, filtration, solar irradiation, straining, and also increases the possibilities of natural death and predation for microbial removal (Gersberg et al. 1989; Williams et al. 1995; Quinonez-D`iaz et al., 2001; Stott et al., 2001; Pundsack et al., 2001; Karim et al., 2004; Stevik et al., 2004; Auset et al., 2005). The increased surface area along with the enhanced physical mechanisms lead to increased active sites for biochemical reactions to happen and increased area for development of bio-film within the treatment bed. And, the performance efficiency of the treatment bed is essentially related to the microbial community present within the bed as removal of contaminants from wastewater are majorly mediated by microbial activities (Reddy, 1983). Thus the microbial community within the treatment bed needs to be regulated in order to enhance the performance of the system for removal of microbial contaminants.

The performance efficiency of wetland systems can also be correlated with the concentration of dissolved oxygen (DO) in the wetland environment; increased bacterial die-off due to increased dissolved oxygen has been already discussed earlier in reported studies (Pearson et al., 1987; Fernandez et al., 1992). The removal mechanism in oxygenic environment is dominated by surface destruction of microbes through oxidation leading to the reduction of oxygen sensitive bacteria (Gross et al., 2007). This effect was dominantly observed for indicator microbial species in the proposed study throughout the monitoring period. As per the results of the study shown in Figure 4.16, higher DO values were observed throughout the treatment bed of *Canna* planted unit, UN-cn as compared to the units UN-ph and UN-ct. This could be due to the high aeration capacity of *Canna indica* roots which create an unfavorable environment for the microbial population and thus leading to higher removal efficiency of microbial contaminants. The high removal efficiency due to high aeration in planted filters is also suggested by Decamp et al. (1999).

The lowest DO values were observed at the middle and outlet points of the unplanted control unit UN-ct, which proves the significance of vegetation in creating an oxygenated environment suitable for higher removal of microbial species. The low DO values in UN-ct is in accordance with the design characteristic of up-flow constructed wetland which have aerobicity at the top followed by anoxic environment at the bottom (Ong et al., 2009; Ghosh and Gopal, 2010). So, the low removal of indicator species from UN-ct might be related to the low concentration of dissolved oxygen.

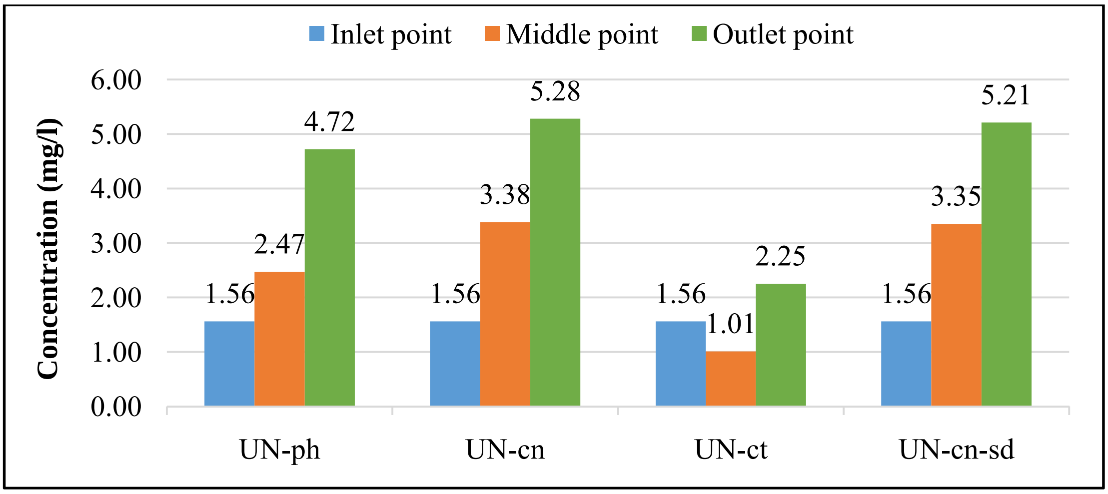

Fig. 4.16. Mean dissolved oxygen (DO) concentrations in mg/l observed for four constructed wetland units at middle and outlet points

A deviation from the above discussion was found when it was observed that the planted unit UN-ph, although having a higher DO concentration as compared to the control unit UN-ct, showed lower performance in terms of removal of indicator organisms. This is in agreement with the previously reported studies by Hench et al., (2003) and Vacca et al., (2005). This result could be justified through the observation by Torrens et al. (2009), who stated that the presence of *Phragmites australis* could be of less significance in vertical flow wetland as compared to the horizontal flow. The probable reason given for the decreased removal from vegetated wetland as compared to the un-vegetated unit was the presence of enteric bacteria and other competing bacteria, as a part of the rhizosphere communities, around the exudates of *Phragmites australis* (Axelrood et al., 1996; Pierson and Pierson, 1996).

Removal of *Salmonella typhi* follows the pattern, UN-ct>UN-cn-sd>UN-ph>UN-cn, different from the pattern shown by the removal of indicator species (Un-cn-sd>UN-cn>UN-ct>UN-ph) which can be explained with the study of other parameters. The lowest removal of *Salmonella* from the unit UN-cn (1.09 log unit) could be related with the lowest removal of COD (34.6%) from the same unit. The low COD removal means enough carbon source is present within the system leading to the increased possibilities for sustainability of *Salmonella* and thus lower removal. Unlike UN-cn, the highest mean removal of *Salmonella typhi* was observed in the control unit UN-ct (2.02 log unit) and this can again be related with highest removal of COD (54.6%). As discussed earlier, high removal of COD from UN-ct could be due to the competition for carbon source between decomposer and heterotrophs, which may contribute to the higher removal of *Salmonella* as limited carbon source might be available for this specie. Moreover, the contribution from plants for carbon source is also not present in the unplanted UN-ct system as compared to the planted units. Thus, it can be concluded that *the removal of Salmonella typhi is highly dependent on the source of carbon present within the system* and it follows the same order as of the COD removal i.e. UN-ct>UN-cn-sd>UN-ph>UN-cn.

4.4.2.5 Improved performance efficiency of UN-cn-sd over UN-cn

The higher removal efficiency of UN-cn-sd over UN-cn can be related to the additional benefits provided through the inclusion of sand layer and more gradation of the media layers in the constructed wetland unit. The inclusion of sand layer helps to maintain the moisture content required for rhizosphere and subsequently increases the contact time between water and treatment bed within the system. The incorporation of sand layer significantly enhances the advantages related to the higher root surface area of *Canna indica* discussed earlier, i.e. enhanced physical mechanisms (aggregation, adsorption, oxidation, sedimentation, filtration, solar irradiation, straining), increased possibilities of natural death and predation for microbial removal, increased active sites for biochemical reactions to happen and increased area for development of bio-film within the treatment bed. The whole system functions in order to provide good aeration and removal of enteric microorganisms sensitive to high concentration of oxygen. The bacterial removal is maximized by the physical mechanisms like retention (filtration/ adsorption) and natural death (Ellis and McCalla, 1976; Reddy et al., 1981; Crane and Moore, 1984). Thus, the systematic increment in the favorable conditions for the removal of microbial contaminants in UN-cn-sd, along with the basic properties of UN-cn, makes it possible to achieve the high removal efficiency of the whole treatment system.

Figure 4.17 clearly indicates that the log removal from Un-cn-sd increased from UN-cn by an amount of 60%, 54% and 45% for *Total coliform, Fecal streptococci* and *Salmonella typhi*, respectively. The results obtained are also in good agreement with studies reported by Vacca et al., (2005) and Sleytr et al., (2007) and are favorable when compared with the study given by Torrens et al. (2007), where less than 2 log unit bacterial removal was observed. Such studies favor the incorporation of sand layer, as a good choice of finer material within the wetland treatment beds, in order to enhance the adherence of microbes; Sleytr et al., (2007) also reported high concentrations of microbes in sediments of sand layer.

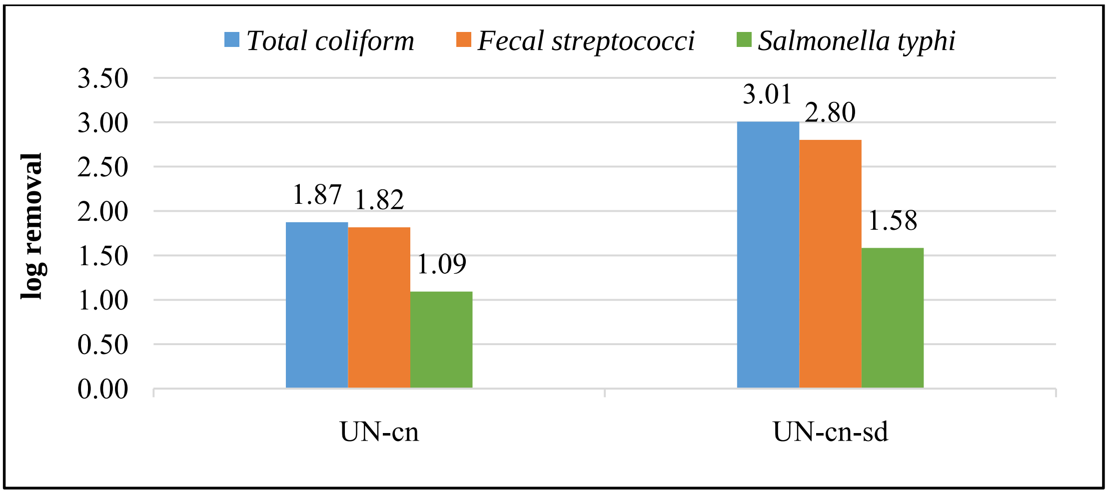

Fig. 4.17. Comparative insight for improved efficiency of *Canna indica* planted units by incorporation of finer media sand

As far as the final effluent concentration of *Total coliform* is of concern, UN-cn-sd was the only unit capable of bringing down the concentration within the prescribed WHO standard limit for safe disposal into water streams; WHO limit is 3 log MPN/100ml and UN-cn-sd achieved 2.75 log MPN/100ml. The results related to UN-cn-sd suggests that it is highly efficient for the removal of all three microbial species. The combined effect of high value of DO in effluent and high removal of COD (low availability of carbon source) lead to the high removal of *Salmonella* by UN-cn-sd. The statistical analysis for *Salmonella typhi* shows that there is no significant difference between UN-cn-sd and UN-ct, and that the difference in the removal from UN-cn-sd was significantly higher than the removal from UN-ph and UN-cn. The results indicate that, although the removal of *Salmonella* is highest in UN-ct, the non-significant difference makes UN-cn-sd the ideal choice for removal of *Salmonella typhi*. It can be elucidate that the *removal or survival of Salmonella typhi is majorly governed by oxygen concentration and carbon source.*

The evaluation of results favors the suitability of *Canna indica* being used with vertical flow constructed wetland for post treatment of secondary treated wastewater in semi-arid climatic conditions prevailing in the region of Rajasthan, India. In such climatic conditions a compact and well-designed vertical up-flow constructed wetland unit, constituting fine material sand along with the coarse material gravel, with gradation, is able to reduce the public health risks associated with the disposal of secondary treated effluents.

4.4.3 Key observations

On the basis of above results and discussions regarding the removal of microbial contaminants, following key observations can be drawn:

1. Significant impact of presence of *Canna indica* on the removal of indicator organisms was observed. The fibrous rooting system of *Canna indica* and inclusion of soil layer improves the aerobicity and physical process within the system, supporting highest performance efficiency of UN-cn-sd.
2. High removal of *Salmonella typhi* might be attributed to the competition between nitrifiers and decomposers for the carbon source (food/nutrient/ substrate) or prolonged exposure to oxygen when the specie is facultative anaerobe.
3. In UN-cn-sd, competition among microbial species for space and nutrients on sand may occur causing higher removal of microbial species.
4. Relatively lower efficiency of UN-ph for indicator organisms can be related to the lower capability of oxygen transportation by *Phragmites australis*, and thus can be related to its lesser significance in the proposed study.

The removal patterns suggest that the mechanisms for indicators and pathogenic species doesn’t greatly differ; in fact it is only the impact of other factors present within particular environment. The physical mechanisms such as sedimentation, adhesion, filtration, adsorption, etc. are more likely to happen in UN-cn-sd followed by UN-cn, UN-ph and UN-ct, in decreasing order. Thus, the cumulative effect of the environmental conditions prevailing within the wetland system show lethal effect on both indicator as well as pathogenic species.

In order to better understand the removal processes occurring in four constructed wetland units, characterization of bacterial community and their respective composition within each of the treatment unit is performed and discussed in the subsequent chapter. Characterization has been performed for biofilm surrounding each of the layers of media as well as root sample of the units. The unit UN-ph is only analyzed for root sample and rest of the three units were characterized at each of the layer of media. All these samples are analyzed through conventional cell-culture dependent technique.

Chapter 5

Bacterial Characterization of Biofilm in Constructed Wetland Units

The results discussed in previous chapter for *Total coliform, Fecal streptococci* and *Salmonella typhi* species give quantitative data analysis on their removal but give no insights into the qualitative analysis of the microbial community structure developed within the constructed wetland units. Qualitative analysis is important as the quality improvement of wastewater is mainly driven by the microbial community through various processes. In order to draw a better understanding for microbial processes driven by microbial assemblage in different treatment units, many authors performed characterization of microbial population for laboratory scale units, for sand filters, and even for full scale constructed wetlands (Ragusa et al., 2004; Vacca et al., 2005; Baptista et al., 2008; Calheiros et al., 2009; Krasnits et al., 2009; Sleytr et al., 2009; Zhang et al., 2010; Dong and Reddy, 2010).

The microbial communities in constructed wetlands are found in the form of biofilm on substrate and root surfaces which plays a major role in improving the water quality through constructed wetlands (Brix, 1994; Stottmeister et al., 2003; Gagon et al., 2007; Kadlec and Wallace 2009). The biofilm in constructed wetlands is formed by bacterial community which is mainly composed of two type of microorganisms - autochthonous (indigenous) and allochthonous (foreign) (Truu et al., 2009). Autochthonous microbes possess metabolic activity to survive and grow in wetland systems and participate in the treatment processes. Whereas allochthonous microbes are pathogens entering into the wetland system through wastewater and they are unable to survive or have any functional importance in the wetland environment (Ansola et al., 2014; Vymazal, 2005).

In the present study the characterization is performed in order to identify the type of bacterial assemblage and their variation with different composition within different constructed wetlands. The study was done for three different rhizosphere to know the change in bacterial assembly with different rhizosphere. Likewise, ten media samples were analyzed to know the changes in bacterial assemblage associated with media of different size from different sites of various wetland units. The observed bacterial species in a sample was first categorized into three phyla - Proteobacteria (P), Firmicutes (F) and Actinobacteria (A) and their further bacteriological analysis was defined in terms of percentage richness distribution and diversity within each sample. The ultimate aim of this bacterial study is to identify the impact of diversified bacterial assemblage on the performance efficiencies of different constructed wetland units.

5.1 Phylotypic Richness Distribution and Diversity in Constructed Wetland Units

Phylotypic percentage richness distribution was calculated as a sum of percentage occurrence of species belonging to a specific phylum and diversity was calculated in terms of number of different species found in given sample (Garbeva et. al., 2004). Thirteen samples were analyzed for bacterial diversity and their relative abundance as phylotypic richness within the constructed wetland units. Three samples were taken from rhizosphere of two plant species from three wetland units, and rest of the ten samples were from corresponding media layers of different constructed wetland units. UN-ph was analyzed only for its rhizosphere since it has the same media distribution as in UN-cn and UN-ct.

Using the culture-dependent method, enumerated bacterial communities were belonged to three different phyla – a) Proteobacteria (*Acinetobacter, Alcaligenes, Citrobacter, Edwardsiella, Enterobacter, Erwinia, Escherichia, Klebsiella, Morganella, Proteus, Providencia, Pseudomonas, Rhizobium, Salmonella, Serratia, Shigella, Yersinia*), b) Firmicutes (*Bacillus, Clostridium, Lactobacillus, Staphylococcus, Streptococcus*) and c) Actinobacteria (*Corynebacterium, Micrococcus*). The retrieved distinct and diverse bacterial communities from the constructed wetland units are in accordance with the reported studies (Cristina et al., 2009; Houda et al., 2014; Adrados et al., 2014; Ansola et al., 2014) where the phylum Proteobacteria was found to be the most dominant followed by Firmicutes and Actinobacteria.

The bacterial characterization results are presented in Table 5.1. The percentage richness distribution of the most dominating phylum, Proteobacteria, predominantly lies in the range of 50-95% followed by lower value ranges of 0-50% for Firmicutes, and 0-14% for Actinobacteria, respectively. Following the same order, diversity was highest for the representatives of Proteobacteria and lies in the range of 2-13 species, followed by a range of 0-3 for Firmicutes and 0-2 for Actinobacteria, respectively.

The total diversity was observed to be highest in the sand sample where 14 types of microbial species were retrieved as compared to the lower diversity in gravel samples. Also, it can be seen from the table that the richness distribution and the overall diversity is mostly affected by the number of representatives of Proteobacteria.

Table 5.1

Richness distribution and diversity of bacterial species of three phyla - Proteobacteria (P), Firmicutes (F) and Actinobacteria (A), enumerated from different samples of each constructed wetland units

Sample	Sample type	Richness distribution (% occurrence)			Diversity (types of species)			
		P	F	A	P	F	A	Total
1	Rhizosphere (UN-ph)	80	20	-	4	1	0	5
2	Rhizosphere (UN-cn)	95	-	5	4	0	1	5
3	Rhizosphere (UN-cn-sd)	85	12	3	7	2	1	10
4	Gravels at outlet (UN-ct)	95	5	-	7	1	0	8
5	Gravels at middle (UN-ct)	82	9	9	7	2	2	11
6	Gravels at inlet (UN-ct)	90	10	-	4	2	0	6
7	Gravels at outlet (UN-cn)	92	8	-	5	1	0	6
8	Gravels at middle (UN-cn)	87	10	3	8	2	1	11
9	Gravels at inlet (UN-cn)	84	12	4	7	1	1	9
10	Gravels at outlet (UN-cn-sd)	79	7	14	5	1	1	7
11	Sand at middle (UN-cn-sd)	94	6	-	13	1	0	14
12	Gravels at middle (UN-cn-sd)	67	22	11	3	3	2	8
13	Gravels at inlet (UN-cn-sd)	50	50	-	2	1	0	3

5.2 Comparative Analysis of Richness Distribution and Diversity among Rhizosphere and Different Media Layers

When comparing the rhizosphere of the three planted units, it has been found that the richness distribution is affected by the plant species as can be seen from the difference in distribution for samples 1 and 2 in Table 5.1, which is enhanced (or better distributed) by the addition of sand layer at rhizosphere of *Canna indica* as evident from sample 3. This enhancement is twice when measured in terms of diversity of bacterial population as shown in the table (total diversity in rhizosphere increased from 5 for UN-cn to 10 for UN-cn-sd). Although, the representatives of the phylum Proteobacteria are dominantly present in all the three rhizosphere samples, there is some distribution of representative species of other phyla as well. The highest diversity observed for the rhizosphere of *Canna indica* planted with sand (UN-cn-sd) is mostly affected by the highest number of representatives of Proteobacteria among the three rhizospheres.

When comparing richness distribution among different media layers in samples numbered from 4 to 13, it was observed that the distribution was not affected by the size of gravels (16-20mm at inlet and 8-12mm at outlet) or their relative location in wetland units, as there was no particular trend observed relative to the media size and phylotypic richness distribution from inlet to outlet or vice-versa. But it was clearly observed that the richness distribution among the three phyla was comparatively higher around the middle zone in all the three units (UN-cn, UN-ct and UN-cn-sd). Similarly, there was no uniform pattern observed for diversity among the media layers within different wetland units; diversity around the outlet zone was highest for the unit UN-ct (sample 4) followed by UN-cn-sd (sample 10) and UN-cn (sample 7), while the diversity found around the inlet zone was highest in case of UN-cn (sample 9) followed by UN-ct (sample 6) and UN-cn-sd (sample 13). Also, the greatest diversity of bacterial species was found to be around the middle zone for all the observed constructed wetland units; diversity at middle zone was found to be always greater than or equal to 8 with highest value of 14 for the sand sample (sample 11). However, when considering the media layers among the three constructed wetland units, with different types of media distributions, it was observed that the unit UN-cn-sd has wider range of diversity (3-14 type of species) as compared to the diversity in the units UN-cn and UN-ct (6-11 type of species). Based on the above comparative analysis of microbial species, in terms of richness distribution and diversity, among the rhizosphere and the media layers of the various constructed wetland units, it can be concluded that the richness distribution and diversity are positively correlated to each other.

Table 5.2 gives the richness distribution and diversity of bacterial species belonging to the three phyla, aggregated for all the media layers within the individual constructed wetland units. It shows that UN-cn-sd has the highest total diversity and high richness distribution. Individually, neither the diversity nor the richness distribution is solely responsible for the overall efficiency of the unit, however, their total and additive effects enhance the treatment efficiency which is supportive of the best performance of UN-cn-sd. Also, in all the wetland units, it can be seen that the highest richness distribution was observed for the species predominantly belonging to the phylum Proteobacteria which were mainly responsible for the performance efficiency of the units.

Table 5.2

Aggregate richness distribution and total diversity of bacterial species of three phyla - Proteobacteria (P), Firmicutes (F) and Actinobacteria (A) for the three constructed wetland units

Constructed wetland	**Plant species / Media distribution**	**Distribution of Richness**			**Total Diversity**
		Proteobacteria	Firmicutes	Actinobacteria	
UN-cn	*Canna indica* / gravels	88	10	2	14
UN-ct	Unplanted / gravels	89	8	3	14
UN-cn-sd	*Canna indica* / gravels + sand	79	16	5	20

*UN-ph was not included in the table due to its same media distribution with UN-cn and UN-ct and no media sampling was done from this unit except its rhizosphere sampling

5.3 Community Composition within Rhizosphere and Different Media Layers

Different microbial species were retrieved from each of the thirteen samples that were given in Table 5.1. As already mentioned, all the microbial species were first categorized into three phyla for the analysis of richness distribution and diversity. The analysis for community composition was then performed in order to investigate the type of microbial assemblages contributing towards the development of biofilm. Table 5.3 gives the insights about the difference in microbial community composition belonging to the three phyla within the rhizospheres of different units. The microbial species identified from rhizosphere samples of the unit planted with *Phragmites australis* (UN-ph) were different from the unit planted with *Canna indica* (UN-cn). Furthermore, many different microbial species were found in the rhizosphere of the unit UN-cn-sd as compared to both UN-ph and UN-cn. It suggests that the presence of sand media plays a significant role to enhance the microbial abundance within the roots of *Canna indica* through providing favorable environment for additional microbial species to grow.

Table 5.4 gives the different types of microbial species present in the media layers which gives insights about the difference in community composition for different media distributions of the wetland units. The different microbial communities in the media samples of the units UN-ct and UN-cn show that there is a similarity of more than 60% of community composition in both the units which can be attributed to the similar media distribution in the two units. It suggests that almost similar type of microbial communities in media are associated with the same type of media distribution regardless of vegetation. And, the different microbial species growth in the vegetated unit UN-cn as compared to UN-ct can be attributed to the presence of rhizosphere in UN-cn. Table 5.4 also shows that the highest number or most diversified microbial communities (20 species) are present in media samples of UN-cn-sd. These observations indicate that the sand media is responsible for the enhancement in community composition as it provides suitable conditions for growth of different microbial communities in wetland system. Hence, the most diversified community structure was observed in *Canna indica* rhizosphere planted with sand layer (UN-cn-sd).

Table 5.3

Community composition of microbial species retrieved from rhizosphere of the constructed wetland units

Phylum	**Microbial spp.**	**% occurrence within rhizosphere samples**		
		UN-ph	UN-cn	UN-cn-sd
Actinobacteria	*Micrococcus spp.*	-	4%	3%
Firmicutes	*Bacillus spp.*	20%	-	9%
Firmicutes	*Streptococcus spp.*	-	-	3%
Proteobacteria	*Alcaligenes faecalis*	-	-	6%
Proteobacteria	*Citrobacter freundii/diversus*	20%	23%	-
Proteobacteria	*Enterobacter aerogenes*	20%	-	-
Proteobacteria	*Escherichia coli*	-	-	6%
Proteobacteria	*Klebsiella pneumoniae*	-	-	12%
Proteobacteria	*Proteus vulgaris*	-	14%	-
Proteobacteria	*Pseudomonas aeruginosa*	20%	-	3%
Proteobacteria	*Rhizobium spp.*	20%	45%	32%
Proteobacteria	*Salmonella spp.*	-	-	3%
Proteobacteria	*Shigella spp.*	-	14%	23%
Total		100%	100%	100%

Table 5.4

Aggregate community composition of microbial species retrieved from all the media layers of the constructed wetland units

Phylum	**Microbial spp.**	**% occurrence**		
		Total media (UN-ct)	Total media (UN-cn)	Total media (UN-cn-sd)
Actinobacteria	*Corynebacterium xerosis*	2%	1%	1%
Actinobacteria	*Micrococcus spp.*	2%	1%	4%
Firmicutes	*Bacillus spp.*	5%	9%	4%
Firmicutes	*Clostridium spp.*	-	-	3%
Firmicutes	*Lactobacillus spp.*	3%	-	-
Firmicutes	*Staphylococcus aureus*	-	1%	8%
Firmicutes	*Streptococcus spp.*	-	-	1%
Proteobacteria	*Alcaligenes faecalis*	-	8%	3%
Proteobacteria	*Citrobacter freundii/diversus*	8%	13%	4%
Proteobacteria	*Edwardsiella tarda*	-	-	3%
Proteobacteria	*Enterobacter aerogenes*	3%	-	7%
Proteobacteria	*Erwinia spp.*	2%	-	-
Proteobacteria	*Escherichia coli*	-	6%	8%
Proteobacteria	*Klebsiella pneumonia*	-	-	9%
Proteobacteria	*Morganella morganii*	2%	-	1%
Proteobacteria	*Proteus vulgaris*	6%	8%	4%
Proteobacteria	*Providencia spp.*	-	2%	1%
Proteobacteria	*Pseudomonas aeruginosa*	8%	2%	3%
Proteobacteria	*Rhizobium spp.*	39%	35%	28%
Proteobacteria	*Salmonella spp.*	-	5%	4%
Proteobacteria	*Serratia marcescens*	3%	3%	1%
Proteobacteria	*Shigella spp.*	14%	6%	3%
Proteobacteria	*Yersinia spp.*	3%	-	-
Total		100%	100%	100%

It is clearly shown that although the number of different bacterial species remain same for UN-cn and UN-ct (Table 5.2), their community composition may differ due to the presence of vegetation (Table 5.3 and 5.4). Such change in community composition might affect the performance efficiency due to specific treatment functions for the various parameters of wastewater study. The observations regarding the effect of vegetation on bacterial assemblage in the study are in accordance with the reported studies by Vacca et al., (2005) and Collins et al., (2004), who reported that the vegetation may affect microbial community composition. Whereas, considering the community composition differences found in the proposed study for planted against unplanted constructed wetland units, it has been observed that certain microbial species like *Lactobacillus spp.*, *Erwinia spp.* and *Yersinia spp.* were only present in unplanted unit UN-ct and not in UN-cn and UN-cn-sd. While certain other species that were absent in UN-ct and present in either UN-cn or UN-cn-sd were *Clostridium spp.*, *Staphylococcus aureus*, *Streptococcus spp.*, *Alcaligenes faecalis*, *Edwardsiella tarda*, *Escherichia coli*, *Klebsiella pneumonia*, *Providencia spp.* and *Salmonella spp.* Hence, vegetation impacts bacterial assemblage or community structure.

Finally, the richness distribution of bacterial communities of the rhizospheres, gravels and sand samples obtained in this study, revealed that the bacterial diversity and its distribution within the three phyla was significantly higher in UN-cn-sd. The difference among the rhizospheres of two plant species in wetland units were found to be more clearly distinguishable than the difference between the gravel layers and sand layer with in a particular unit. This observation is in agreement with the study reported by Sleytr et al., (2009). The enhanced bacterial diversity of UN-cn-sd is in accordance with the studies by Smella et al., (2001) and Kowalchuk et al., (2002), where influence of plant species on bacterial diversity in rhizosphere in presence of sand media was reported. The highest range of diversity in UN-cn-sd could be related to its overall best performance resulted by synergistic effects of all three phyla richness distribution due to the presence of sand as well as *Canna indica* vegetation. Greatest diversity was found to be around the middle zones, irrespective of the different vegetation and media size/distribution, and suggested that the location have a strong effect on bacterial community as found in the study by Iasur-Kruh et al., (2010). While comparing media with sand layer and without sand layer, it was clearly indicated that there is significantly higher representatives of Proteobacteria along with the highest diversity in the sand sample. This observation is in accordance with the study by Calheiros et al., (2009) where the porous matrix was observed to provide the greater surface area for treatment contact and biofilm development.

Chapter 6

Summary, Conclusions and Recommendations for Future Work

This chapter presents the conclusions of the study against the objectives suggested in Chapter 2. Based on the inferences and outcomes of the research, following conclusions can be drawn (the major findings related to the microbiological contaminants removals are presented first, followed by conclusions drawn from the physico-chemical analysis):

1. Among the initially developed units (UN-ph, UN-cn and UN-ct), the *Canna indica* planted unit (UN-cn) showed the highest removal efficiency in terms of indicator organisms - 1.87 log removal for *Total coliform* and 1.82 log removal for *Fecal streptococci*. Thus, results indicate that the impact of presence of *Canna indica* for indicator organisms is significant. However, the effectiveness related to *Canna indica* did not greatly affect the organic matter removal where UN-cn showed lowest removal of COD with 34.6%. The highest removal of *Salmonella typhi* was shown by UN-ct with 2.02 log removal, whereas removal from UN-cn was lowest with 1.09 log removal. The lowest removal of *Salmonella typhi* is possibly related to lowest removal of organic matter in the UN-cn, whereas, the highest removal of *Salmonella typhi* might be linked with the highest removal of COD (54.6%) in control unit UN-ct. These observations clearly show that the removal of *Salmonella spp.* might be dependent on the availability of food/carbon source within the system (Ponugoti et al., 1997). This indicates that the competition for the substrate might be the cause of the increased removal of *Salmonella* from control unit.

2. In order to achieve the prescribed limit for *Total coliform* (1000MPN/100ml) for safe disposal into water streams, a unit of constructed wetland (UN-cn-sd) with incorporation of sand layer showed highly enhanced efficiency in terms removal of *Total coliform* (3.01 log removal), *Fecal streptococci* (2.80 log removal) and *Salmonella typhi* (1.58 log removal). Although, the mean removal of COD and *Salmonella typhi* from UN-cn-sd was lower than the corresponding removal observed from UN-ct, there was no significant difference (p-value>0.05) found in the log removals of both units, which confirms the conclusive significance of the sand incorporated unit, UN-cn-sd.

3. Higher microbial diversity in the unit UN-cn-sd is indicative of improved treatment process within the system of this unit and it helps to perform functions much similar to the natural conditions. Highest diversity around sand may have caused the competition for space and nutrients (evident from high organic matter and nutrient removal), further contributing to the increased removal of *Salmonella typhi*. Representatives of Proteobacteria are mainly responsible for the removal mechanisms including synergism with Firmicutes and Actinobacteria.

4. According to the results, it can be elucidated that *Canna indica* was found to be a suitable plant species for removal of microbial contaminants from secondary treated wastewater, in semi-arid climatic conditions. However, the incorporation of sand bed was found to be effective to meet the prescribed disposal standards for *Total coliform*. Enhanced performance of UN-cn-sd may be related to the combined effect of dominance of fibrous root pattern of *Canna*, high aerobicity, competition for food and space, increased physical mechanisms, higher microbial diversity along with higher community richness (and resulted high number of active site for bio-chemical processes). No single mechanism can govern the performance of any system.

5. In India, there are no prescribed standards for microbial contaminants in the treated disposals, and hence there is a need to set permissible levels for microbial contaminants. This research shows that the compact constructed wetlands could serve such facilities to achieve the microbial contaminants level within the standards prescribed by WHO i.e. 1000 coliform per 100 ml.

6. Presence of plant and use of different plant species did not greatly affected the removal of organic matter. Based on the observed results, this can be elucidated that the organic matter removal occurs mainly due to the total effect of different biochemical and physical processes (filtration, sedimentation, adsorption, etc.) that were enhanced in the unit UN-cn-sd.

7. High removal of COD from UN-ct is due to the simultaneous cumulative effect of oxidation and heterotrophic denitrification which causes increased utilization of oxygen and carbon source. However, the removal of nutrients in this unit could be due to the basic characteristic design of vertical up-flow

regime where oxic and anoxic conditions prevailed and corresponds nitrification and heterotrophic denitrification including ANAMMOX in the respective zone.

8. *Canna indica* is found to be the best suitable plant species for nutrients removal and this can be enhanced by creating gradation effect through inclusion of finer material, such as sand in UN-cn-sd, which helps in enabling the higher porous system. The dominant processes related to the nutrients removal was nitrification, the availability of high dissolved oxygen concentration and higher removal of ammonia-N. While comparing the concentration of dissolved oxygen in all wetland units, it was clearly found that the plants have significant capacity to transport oxygen throughout the treatment system of vertical flow regime.

Recommendations for Future Work

The proposed study achieved its objective to investigate the performance efficiency of four constructed wetland units in reducing the microbial contaminants from secondary treated wastewater. But due to the limitations related to the availability of resources and time frame, the study has revealed a range of issues which are required to be investigated on the priority basis.

1. The present research is significant in both practical and engineering terms for the treatment of secondary treated effluent in semi-arid conditions where the public health is the important priority. In this proposed study, the constructed wetland was planted with two different species and *Canna indica* was found to be more suitable than *Phragmites australis* in treating effluent. However, the comparative study of *Canna indica* with other locally available plant species should/can also be carried out.

2. Further research is recommended to pursue this study more accurately in other regions with different environmental conditions to assure the significant of use of *Canna* plant species in improving the quality of secondary treated wastewater. Otherwise, it cannot be strongly recommended unless the detailed experimental examination is done.

3. UN-cn-sd is proposed as the suitable media configuration and plant species on the basis of above mentioned conclusions. The proposed research is based on the results obtained by laboratory scale experimental set up. In order to improve the quality of secondary treated wastewater, it is recommended to pursue the same research at pilot scale on site as a tertiary treatment step.

4. During the analysis of removal mechanism of indicator and pathogenic species, the removal mechanism was found to be related to the increased physical mechanism as well as the concentration of dissolved oxygen. Whereas the dependency of *Salmonella* spp. was observed for carbon source including dissolved oxygen concentration. This dependency of *Salmonella* spp. on carbon source should be examined in detail using different doses.

5. The proposed construed wetland configuration (UN-cn-sd) is suggested for ASP based treatment plant as a tertiary treatment step, but the efficiency should also be checked with effluent from different types of wastewater, such as effluent from UASB.

6. It has been well documented that UN-cn-sd was found to be a suitable wetland model for reduction of microbial species for both indicator and pathogenic species. So this unit needs to be optimized by using different media sizes and different height of media layers. The optimization should also include the constructed of control unit (unvegetated) corresponding to the UN-cn-sd which would be able to give more specific information regarding the role of sand layer.

7. The hydraulic study should be done for different retention time where the proposed study is only based on 2-3 day HRT. Moreover, this study needs to be investigated for long term analysis due to limited source availability of the proposed study.

8. The cost estimation of the proposed technology should also be taken into consideration, along with its comparison with other conventional wastewater treatment techniques.

Moreover, the research outcomes of this study may be incorporated as a significant and valuable addition in the existing knowledge. This should be taken into consideration with Government authorities when tertiary treatment steps after sewage treatment plants (STP) are of concern.

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List of Publications

Following is the list of research articles published, confirmations are enclosed here:

- Priya, Gargi Sharma and Dr. Urmila Brighu (2013). Comparison of Different Types of Media for Nutrient Removal Efficiency in Vertical Upflow Constructed Wetlands. International Journal of Environmental Engineering and Management, 4, (5), 405-416

- Gargi Sharma, Priya and Urmila Brighu (2014). Performance Analysis of Vertical Up-flow Constructed Wetlands for Secondary Treated Effluent. APCBEE Procedia, 10, 110 – 114

- Gargi Sharma and Urmila Brighu (2016). Selection of suitable plant species in semi-arid climatic conditions for quality improvement of secondary treated effluent by using vertical constructed wetland. Nature Environment and Pollution Technology,15, (1), 325-329

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Performance Analysis of Vertical Up-flow Constructed Wetlands for Secondary Treated Effluent

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Abstract

The use of constructed wetlands for wastewater treatment has been exercised since 1950's and still are being in use. The vertical flow constructed wetlands provide more oxygenated environment and significantly reduce the organic matter as well as microbial species from wastewater. In the present study vertical up-flow constructed wetlands were constructed and used as bio-filter to improve the water quality of secondary treated effluent. The reduction pattern is studied in this research and correlated with plant species and presence of plant. The plant species used in the constructed wetlands were *canna* and *phragmitis*. The fibrous rooting system of *canna* species causes the high aerobic conditions throughout the treatment bed which in turn facilitates higher removal in comparison to *phragmitis* planted wetland. Removal of nitrogenous compounds like ammonia-nitrogen, TKN and nitrate were observed better in *canna* planted wetlands than others.

Selection and peer review under responsibility of Asia-Pacific Chemical, Biological & Environmental Engineering Society

Keywords: Constructed Wetland, *Canna Indica*, *Phragmitis australis,* Secondary treated wastewater

1. Introduction

In India, the availability of large land area is prime constraint for establishment of field scale constructed wetlands. While the subsurface vertical flow systems generally associated with about a 100 times smaller size range and 3 times smaller HRTs than the surface flow. Therefore, the vertical flow constructed seem to have an implication for better acceptability under Indian conditions [1], as it has been proven effective to treat

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Original Research Paper

Selection of Suitable Plant Species in Semi Arid Climatic Conditions for Quality Improvement of Secondary Treated Effluent by Using Vertical Constructed Wetland

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Canna indica
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ABSTRACT

The wetland plant species play a critical role in determining the performance of the wetland systems. Thus selection of suitable plant species for vegetation in treatment wetland units is of great importance to enhance the efficiency of the system. The present research aims to identify the suitable plant species for constructed wetlands in the semi-arid climate of Rajasthan (India). The performance of the two widely used Indian wetland plants, *Phragmitis australis* and *Canna indica* were evaluated in vertical up-flow constructed wetland using secondary treated effluents. Performance efficiency of both the plant species was evaluated for physico-chemical and microbial contaminants removal. The proposed study highlights the comparative as well as significant suitability of *Canna indica* plantation over the *Phragmitis australis* under semi-arid climatic conditions. The unit planted with *Canna indica* showed 39.7 and 50.9% removal for total Kjeldahl nitrogen (TKN) and ammonia-nitrogen respectively. Nitrate nitrogen in the treated effluents has a significant increment of 3.8 times higher than influents. Importantly, the indicator organism coliform reduction was observed as 1.87 log (MPN/100mL) in the effluent of *Canna indica* planted unit as against 1.01 log (MPN/100mL) in the effluent of *Phragmitis australis* planted wetland.

INTRODUCTION

In recent decades, planned reuse of wastewater has gained importance, as the demand for water dramatically increased due to increased population growth, urbanization and technological advancements (Vigneswaran & Sundaravadivel 2004). As a part of urban planning and management, in India, government has developed the technically sound centralized wastewater treatment plants in every state of the country. Their effluent quality is regulated by imposing standards for their corresponding final disposal (discharge into water bodies and its reuse). But, it has been clearly stated in the report of Central Pollution Control Board, India that the effluent from treatment plants rarely meets the standard limits of final disposal (CPCB 2008). Therefore, a tertiary treatment step has been suggested and employed in many of the treatment sites.

The technologies like chemical dosing, biological treatment using constructed wetlands and disinfection by UV and others are being used at this step. Constructed wetland is the most popular environmentally sound option and its suitability has been proved for removal of the contaminants since 1950s. The treatment plants are conventionally based on physico-chemical contaminants as well as nutrient removal only. There is lack of strict national standards for microbial contaminants in effluent disposal; the research is an attempt to improve the quality of secondary treated effluent in Jaipur, Rajasthan using constructed wetlands. Constructed wetlands technology has been used to improve the effluent quality especially in terms of microbial contaminants removal. Large land availability is prime constraint in India for developing field scale constructed wetlands, therefore, vertical upflow constructed wetland (VUFCW) was selected within the aim of the study. The research is firmly based on literature which clearly indicated the significant use of constructed wetlands for microbial contaminants removal (Hench et al. 2003, Vacca et al. 2005, Sleytr et al. 2007, Kadam et al. 2008, Chang et al. 2010). The challenge was to select suitable, efficient and effective plant species that are available in semi arid climatic conditions prevailing in Rajasthan, India. However, the use of *Phragmitis* plant species for wetland establishment is a tradition and still in practice worldwide. *Canna indica* is a well studied plant species for wetland establishment in China and other countries (Calheiros et al. 2007, Naz et al. 2009).

The present research is aiming to investigate the best suitable plant species while comparing *Phragmitis australis* and *Canna indica* for secondary treated effluent for removal of coliforms and other contaminants.

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Comparison of Different Types of Media for Nutrient Removal Efficiency in Vertical Upflow Constructed Wetlands

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Abstract

This study investigated the efficiency of two types of media, namely, gravels and sand for their nutrient removal capabilities from wastewater. Different levels of sand depths were also experimented for their removal efficiencies. And for this purpose three laboratory scale vertical upflow constructed wetlands (S1, S2, S3) were established at PHE Laboratory, MNIT, Jaipur. S1was filled with two layers of different size of gravels while S2 and S3 also contained different depths of sand along with the layers of gravels. All the lab scale CWs were fed with the secondary treated water brought from sewage treatment plant, Jaipur. The study was carried out for a period of three months from February to April, 2013. The results show that sand provides better removal of nutrients from wastewater than gravels, though TKN removal was better with gravels. Both sand and gravel were unable to remove NO_3-N from the system as there was an increase in NO_3-N in the system. But overall it can be concluded that sand provided more efficient treatment than gravels.

Keywords: Vertical upflow constructed wetlands, nutrient removal, sand, gravel, Canna indica, tertiary treatment.