COMPARISON OF INFLUENT AND EFFLUENT WATER QUALITY OF MEMBRANE BIOREACTOR (MBR) AND PHYTOREMEDIATION PLANT

By

Khush Bakht Andleeb

(Reg#00000118139)

A thesis submitted in partial fulfillment of requirements for the degree of

Master of Science

In

Environmental Sciences

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

(2018)

It is certified that the contents and forms of the thesis entitled

COMPARISON OF INFLUENT AND EFFLUENT WATER QUALITY OF MEMBRANE BIOREACTOR (MBR) AND PHYTOREMEDIATION PLANT

Submitted by

Khush Bakht Andleeb

Has been found satisfactory for the requirements of the degree of

Master of Science in Environmental Sciences

Supervisor: ________________

Dr. Imran Hashmi

Professor

IESE, SCEE, NUST

Member: __________________________

Dr. Sher Jamal Khan

Associate Professor

IESE, SCEE, NUST

Member: __________________________

Dr. Hamza Farooq Gabriel

Professor

NICE, SCEE, NUST

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS thesis written by Ms. **Khush Bakht Andleeb,** Registration No. **00000118139** of **IESE (SCEE)** has been vetted by undersigned, found complete in all aspects as per NUST Statutes/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/MPhil. Degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

Signature with stamp: ____________________________

Name of Supervisor: _____________________________

Date: ____

Signature of HoD with stamp: ______________________

Date: ____

Countersigned by

Signature (Dean/Principal): _________________________

Date: ____

This Thesis is dedicated to Ami Abbu whose continuous support and prayers are always with me whenever and wherever required

ACKNOWLEDGEMENTS

All praises to Allah for His countless blessings and strengths in completing this thesis. First and foremost I would like to express my gratitude to my supervisor Dr Imran Hashmi for his consistent support, appreciation and motivation throughout my research work. His invaluable help of constructive comments and suggestions throughout the experimental and thesis work have contributed to the success of process. I would like to thank my Guidance and Examination Committee (GEC) ***Dr. Sher Jamal Khan,*** *Associate Professor, IESE, SCEE, NUST and* ***Dr. Hamza Farooq Gabriel,*** *Professor, NICE, SCEE, NUST for their constant support and knowledge wherever required.*

I would like to express my warmest and deepest appreciation to Lab demonstrator Madam Sahaab Farooq, who is always there for guidance, help and encouragement during this vigorous work.

I owe profound gratitude to my seniors Ms. Romana Khan (IESE), Ms. Habiba Daud (IESE) and Mr. Danish Gul (ASAB) for their dedicated interest in my research activities.

Most importantly I would like to thanks my Brothers; who mean the world to me and my Sister in Law, for always being so supportive and encouraging.

Last but not the least, my dear friends; Sadia Sami, Sana Nisar, Shagufta Irum, Rabia Akmal and Amrah Qureshi, who were always being so loving and caring.

KHUSH BAKHT ANDLEEB

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
BOD	Biological Oxygen Demand
CFU	Colony Forming Unit
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
EC	Electrical Conductivity
EMB	Eosin Methylene Blue
GHI	Global Horizontal Irradiance
MBR	Membrane Bioreactor
mg/L	Milligram Per Liter
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
µS/cm	Microsiemens Per Centimeter

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Inadequate sanitation and unavailability of clean water have become the most pervasive problems affecting people throughout the developing world. Conventional wastewater treatment technologies have proved ineffective in solving complex water related issues resulting from rapid industrialization and urbanization throughout the developing world. Membrane bioreactors (MBR) and Phytoremediation system under investigation have showed to be very robust Eco technologies for domestic wastewater treatment and reuse. The present study was conducted at NUST H-12 campus Islamabad to compare the water quality of MBR and Phytoremediation system in terms of Physico-chemical and Biological parameters and microbial species present in the system. Phytoremediation system under study was planted with *Typha latifolia, Pistia stratiotes, Centella asiatica*. Higher treatment efficiency was achieved (up to 90.8% for TSS, 67.7% for COD, 95% for turbidity and 90% for total coliform) by Phytoremediation system. Treatment performance of MBR system was up to 99% for TSS, 83% for COD, 91.5% for turbidity and 99% for total coliform and final effluents proved to compile with the EPA regulations. The high removal rates were achieved at higher temperature as well as other meteorological parameters (wind speed, air pressure, relative humidity, global horizontal irradiance) showed a significant positive and negative correlation with the removal efficiencies. Elemental uptake by plants was exhibited higher in summers than winters. 97.9% decrease in relative growth rate of *Pistia stratiotes* was observed followed by 91.88% for *Typha latifolia* and 74.5% for *Centella asiatica*. Predominant species isolated and identified from wastewater of Phytoremediation system belong to the phyla Proteobacterium (*Enterobacter cloacae*, *Enterobacter kobei*, *Enterobacter hormaechei, Enterobacter asburiae, Enterobacter aerogenes. gamma proteobacterium, Franconibacter pulveris, Citrobacter freundii, Shigella dysenteriae, Escherichia albertii* and *Escherichia coli)*. While, predominant bacterial species isolated and identified from activated sludge of MBR system were *Salmonella enterica, Pantoea dispersa, Shigella dysenteriae, Enterobacter hormaechei* and *Salmonella waycross* and they too belong to the phyla proteobacteria.

Key words: *Membrane bioreactor (MBR), Phytoremediation system, Physico-chemical parameters, Biological parameters, Meteorological parameter*

1. INTRODUCTION

1.1 Background

Covering about 70% of the earth's surface, water is recognized as a most valuable resource that exist on our globe. Inadequate provision of safe and clean water has become one of the most prevalent problems in developing countries and problem is expected to rise in the coming years. According to a report by WHO (2012) more than 780 million people of the world which is about one tenth of the world population still depend on low quality drinking water in 2010 and about 2.5 billion people around the globe still have least access to the improved sanitation. To meet the rising water demands people are overexploiting the natural ground water resources that are resulting in various environmental consequences like ground water subsidence and ecosystem deterioration (Orebiyi & Awomeso, 2008; Zhang *et al.*, 2014).

Water is referred to as polluted, when it is impaired by the release of anthropogenic contaminants. The resulting polluted water undergoes a mark shift in its ability to support its affiliated biotic communities as well as does not remain portable for human use. Majority of the water pollutants are being carried by rivers into the longer water bodies, ultimately making them impure and posing risks to the human health (Sharma & Dubey, 2011).

Water pollution has resulted in many problems all over the world which include drinking water supply, sanitation supplies and survival of the biotic species. Direct water pollution refers to the release of pollutants from refineries, factories, sewage treatment plants, directly into the urban water provisions while indirect pollution refers to the addition of contaminants in the drinking water supply from ground/soil water system and from the atmosphere through rain water. Some major pollutants found in water include organic matter, metals, xenobiotics, nutrients and acidic gases such as sulphur dioxide. Discharge of pollutants from domestic and industrial sources has detrimental effects on the aquatic ecosystem as this can result in deposition of large amount of nutrients, organic matter and pollutants leading to eutrophication, oxygen deficiency in the aquatic ecosystem and deposition of pollutants in the receiving water bodies (Wakelin *et al.*, 2008).

Four main types of wastewater have been identified, namely domestic, industrial, agriculture and storm water. Urban wastewater is defined as a combination of domestic and industrial wastewater as well as surrounding sewage infiltration and rain water whilst agricultural wastewater consists of wastewater generated through processes from surrounding farms, agricultural activities and sometimes contaminated groundwater (Choukr-Allah & Hamdy, 2005). Following are the categories of wastewater:

1.2 Characterization of wastewater

Table 1.1 categorizes the types of wastewater along with its sources and effects

Table 1.1: Categorization of wastewater

Type of wastewater	Sources	Effects
Domestic wastewaters	Comprises of wastewater from daily personal use, laundry, and human wastes.	Domestic waste contains disease causing pathogens that are responsible for number of diseases like typhoid or cholera.
Industrial wastewater	Comprises of byproducts of commercial and industrial activities.	Major concern of these wastes is the chemical reaction that may occur with the environment because of presence of toxic chemicals that consume oxygen from atmosphere.
Storm water	Refers to the runoff from urban and agricultural areas such as parks, roofs, gardens, paths, roads and gutters into drains after rain.	These wastes are harmful for fish and other aquatic life and even kill them. Furthermore, make waterways unhealthy place to work, live and play.

1.3 Pollution due to domestic wastewater

Domestic wastewater is defined as the water that is used by a community and contains all the constituents added to the water during its consumption. It is thus comprised of human and animal wastes along with the water used for laundry, personal washing, cooking, bathing, utensils cleaning etc. Fresh wastewater is a turbid liquid of gray color that having inoffensive odor. It comprises of dissolved and suspended solids along with other harmful pollutants. It is obnoxious in appearance and contain hazardous content, because of the presence of number of pathogenic organisms it contains. Domestic wastewater comprised of number of chemicals such as fats, grease, detergents, soaps, any rotten food that pass from kitchen sink, soil particles and sand etc. (Hülsen *et al.,* 2016)**.**

In addition to multiple chemical compounds it also contains many masses of intestinal bacteria and other smaller numbers organisms. Domestic waste also contributes varied variety of chemicals such as soaps, fats and grease, detergents and pesticides, including anything that passes out from kitchen sink, such as vegetable peelings, sour milk, soil particles and sand etc. The list is so extensive that it’s impossible to quantify each and every element (Micheal *et al.*, 2009).

1.4 Water crises in Pakistan

Per capita annual water availability of Pakistan has decreased from 5000 cubic meter in 1951 to 1038 cubic meter in 2010 which is slightly higher than the internationally acceptable water scarcity level of 1000. According to report by International Monetary Fund (IMF), Pakistan is now designated as the third most water-stressed country globally because its per capita yearly water availability is 1,017 m^3. This is a clear indication that Pakistan has the most intensive water economy in the world. In addition, water bodies are polluting due to discharge of untreated wastewater into fresh water bodies and is affecting this already vulnerable resource (Haydar *et al.*, 2015).

The main reason attributed for this cause is rapid population growth and urbanization. The issue is also worsened by poor water management climate change, and a lack of political stability. The water scarcity issues are promoting conflicts in the country. Economic impact of crises is huge and people are fighting for the basic resources.

1.5 How to cope with this crisis?

In order to cope with the water crises, effective water management strategies should be adopted to reuse wastewater in a sustainable way. Variety of wastewater treatment technologies are there to treat wastewater. The detail of which are mentioned below:

1.5.1 Wastewater treatment technologies

Inadequate approach to clean water and hygiene has turned into one of the most persistent issues afflicting public throughout the developing world. Imitation of centralized energy, water and cost effective technologies have proved useless in solving the intricate water linked problems resulting from quick urbanization in the developing countries and problems with water are expected to worsen in coming decades (Fountoulakis *et al*., 2017).

Among the diverse developments, treatment of wastewater in developing countries is always considered one of the lowest priorities. The consequence of this is the common practice of discharging large amounts of untreated wastewater directly into streams and lakes in many developing countries (Vymazal & Březinov, 2014; Mander *et al*., 2014).

There are physical, chemical and biological methods used to remove contaminants from waste-water (Figure 1.1). In order to achieve different levels of contaminant removal, individual waste-water treatment procedures are combined into a variety of systems, classified as primary, secondary, and tertiary waste-water treatment. More rigorous treatment of waste-water includes

the removal of specific contaminants as well as the removal and control of nutrients. Natural systems are also used for the treatment of wastewater in land-based applications. Sludge resulting from wastewater treatment operations is treated by various methods in order to reduce its water and organic content and make it suitable for final disposal and reuse (Mara *et al.*, 2013).

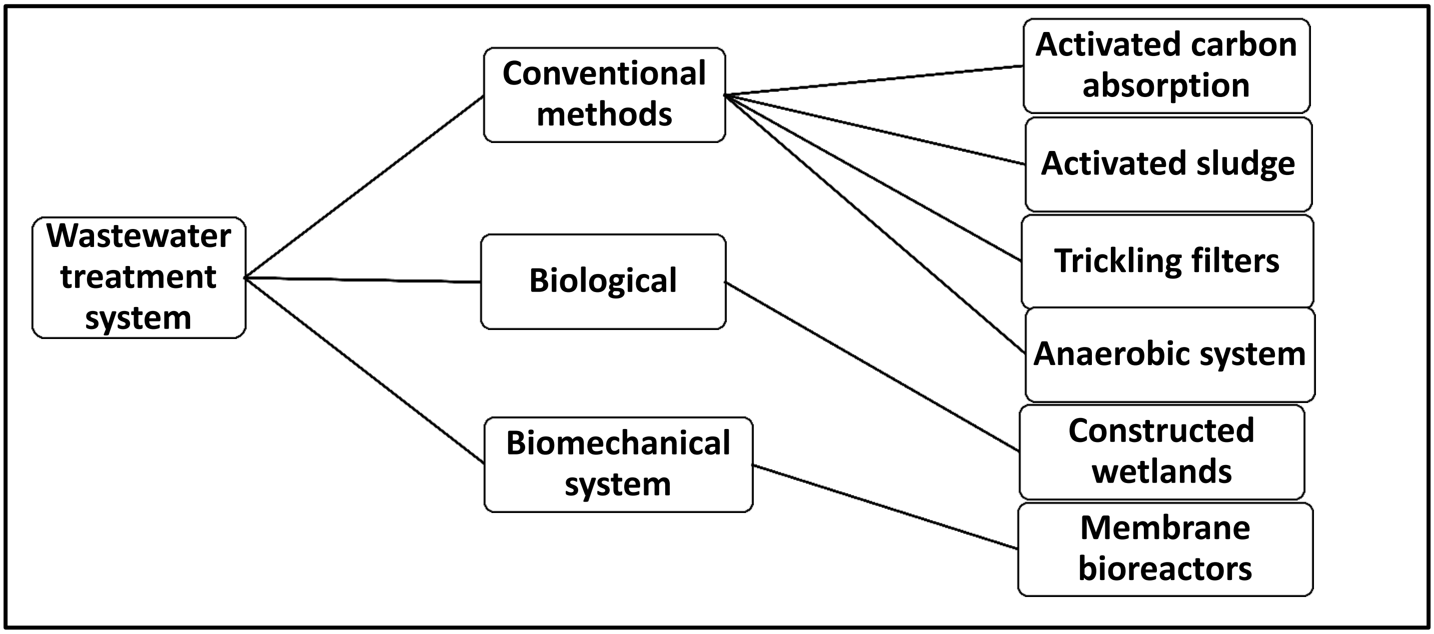


Figure 1.1: Characterization of wastewater treatment technologies

Constructed wetlands are natural (biological) wastewater treatment systems that are becoming an important alternative to conventional wastewater treatment systems because of its efficiency, less establishment and management requirements (Adrados *et al*., 2014; Luo *et al.,* 2016). Membrane bioreactor technology (biomechanical) combines membrane filtration and biologically activated sludge process and has been recognized as a most popular, abundant and efficient technology in recent years for treatment of wastewater (Hashisho and El-Fadel, 2016; Wang *et al*., 2016)

Removals of pollutants by Constructed wetlands depend upon various factors including absorption, filtration, sedimentation, uptake by plants and various microbial processes. These processes are influenced by various factors like temperature, redox conditions, soil type, feeding mode, design of setup and types of macrophytes involved. Major role of macrophytes involved in system is to provide suitable state for physical purification, stabilization of surface beds and provision of increased surface area for microbial growth (Wu *et al.*, 2014; Oon *et al.*, 2015).

Due to rapid increase in population, conventional wastewater treatment plants have become overloaded and there is less space left for extension of existing treatment systems. In view of this **MBR technology** emerged as an efficient technology with less sludge production, smaller ecological footprints and better treated water quality. Highly sensitive membrane is able to remove micropollutants from wastewater (Wang *et al.*, 2016; Neoh *et al*., 2016). However, Constructed wetlands are suitable in terms of capital cost, annual running cost, less electricity and technically trained human resources requirements and less maintenance requirements. Capital cost of CWs and MBR system is approx. 62000 $ and 390000 $ respectively while annual running cost of Constructed wetlands and MBR system is approx. 4428$ and 10,000$ respectively (Neoh *et al*., 2016).

1.6 Present study

The study was conducted to compare the performance efficiency of pilot scale Phytoremediation and MBR system and to compare the microbial diversity present in surface and sediments of Phytoremediation plant and activated sludge of MBR system. Further total biomass of Phyto remediating plants was estimated and relative growth rate was measured.

1.7 Aims and objectives

- Comparative evaluation of treated water of MBR plant and phytoremediation plant.
- Comparative characterization of microbes of surface and sediments of wetlands and activated sludge of MBR system.
- Total biomass estimation of predominant macrophytes species.

2. LITERATURE REVIEW

2.1 Wastewater treatment

Rapid expansion in urbanization and industrialization is continuously exerting pressure on limited resources of the planet, particularly fresh water resources. The diminishing of clean water resources has urged the necessity to search for unconventional means to complement fresh water resources. With the development of concept of sustainable development that was introduced in 1992 at Rio Summit; Reduce, reuse and recycle were the suitable norms adopted for the resource conservation.

2.1.1 Conventional wastewater treatment technologies

Some of the dominant conventional wastewater treatment technologies include septic tanks, trickling filters, activated sludge, activated carbon absorption and stabilization ponds. These treatment technologies have greatly upgraded the quality of wastewater being released into environment. Introduction of innovative and advanced treatment technologies are required to replace the conventional treatment technologies that are both energy intensive and less efficient. The basic goal is to achieve resource conservation through recycle and reuse strategies (Vigneswaran & Sundaravadivel, 2014).

Conventional wastewater treatment includes various steps:

- **Primary treatment:** It involves controlling the velocity of incoming wastewater through grit channels to settle down large objects that make their way into the sewerage system. Also, bar screens are used to remove large objects.
- **Secondary treatment:** It involves removal of organic matter up to 90% by use of biological organisms that involves suspended growth process and attached growth process.

- **Tertiary treatment:** It involves advanced removal of dissolved substances such as metals, organic compounds, nutrients etc. followed by multiple advanced treatment processes (Rashidi & Hoseni, 2015).

2.1.2 Advanced wastewater treatment technologies

To treat water to the level at which it can be reused, number of technologies as alternative are introduced in the recent era to overcome the water crises issues and they are successfully adopted in some areas as well.

These wastewater treatment technologies include:

- Biological processes for tertiary treatment of wastewater for organic matter and nutrient removal. It includes Constructed wetlands (CWs), which are biologically enhanced phosphorus, nitrogen and organic matter removal (BEPR) systems. They are recognized as an important biological treatment technologies.
- Physicochemical methods such as membrane filtration and deep bed filtration.
- Hybrid procedures such as amalgamation of physicochemical and biological progressions as in membrane bioreactors (MBR) (Vigneswaran & Sundaravadivel, 2014).

2.1.2.1 Biological processes (Constructed wetlands)

- **Definition and classification**

Constructed wetlands are the engineering systems which are designed in a way so that it mimics the natural system of wetland for treating different types of wastewater including domestic and industrial wastes. It mainly comprised of the vegetation specific for pollutants removal, soil, microorganisms and water and involves variety of complex chemical, physical and biological processes to enhance the water quality and reuse it for various purposes (Vymazal, 2011; Saeed and Sun, 2013).

Scheme of various types of constructed wetlands is shown in Figure 2.1. Based on the hydrology of wetlands it is divided into two types:

- Free water surface (FWS) Constructed wetlands (CWs)
- Subsurface flow (SSF) Constructed wetlands (CWs)

FWS wetland systems are analogous to natural wetlands which involves the shallow flow of water over saturated substrate. In SSF wetland systems, water usually flows vertically or horizontally through the substrate which promotes the growth of macrophytes, and based on the flow direction, SSF CWs can be further divided into vertical flow and horizontal flow CWs.

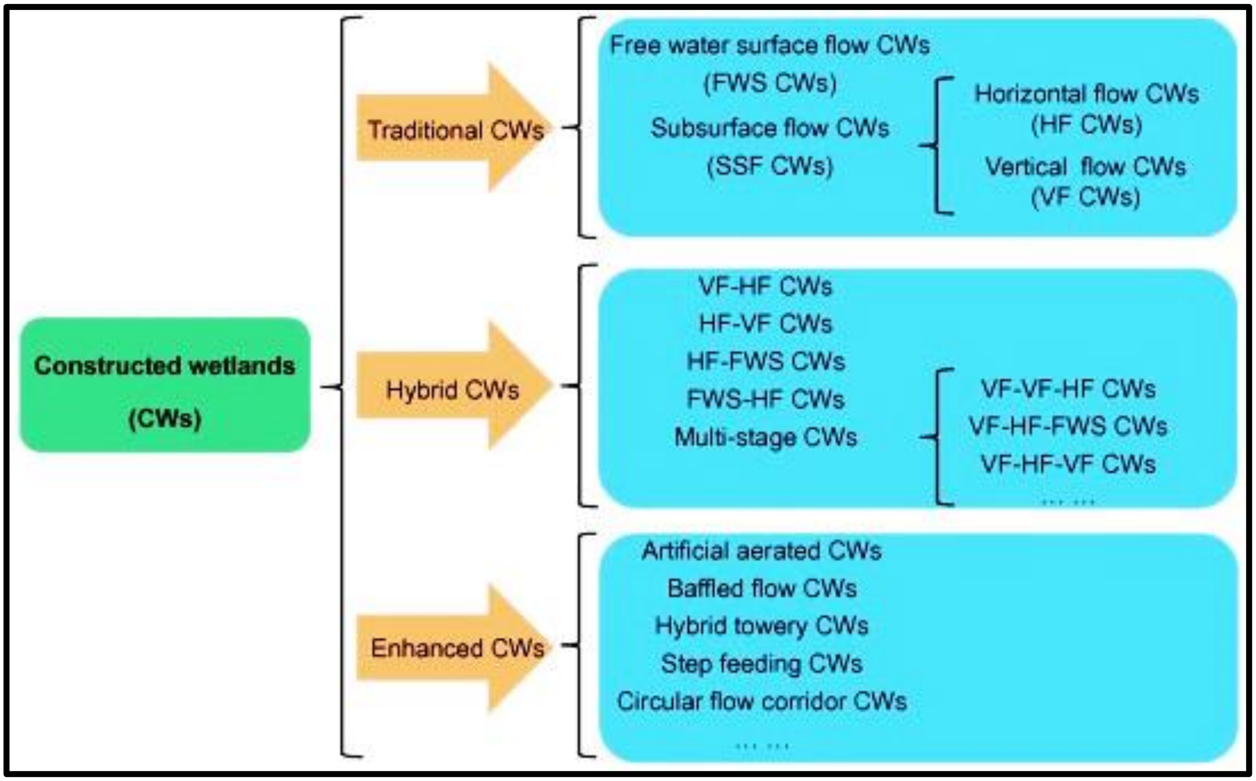


Figure 2.1: Schematic diagram of classification of constructed wetlands (Saeed and Sun, 2013).

- **Brief history**

The first effort intended to the possibility of Constructed wetlands for wastewater treatment was implemented by Käthe Seidel of Germany in the early 1950s after that experiments on CWs were carried out and applied for wastewater treatments successively in the 1960s and 1970s. At the start of experimental phase, CWs were mainly used for treating municipal and domestic wastewater. But now application of this treatment systems has been extended to the treatment of industrial and agricultural efflents, landfill leachate, mine drainage, polluted lakes and rivers, urban runoff and it is implemented in various climatic conditions around the worlds such as tropical, arid and sem arid regions, hot and humid climate (Wu *et al.,* 2014). Full-scale CWs were constructed during the late 1960s and now there are more than 10,000 CWs in North America and 50,000 CWs in Europe (Kadlec and Wallace, 2009; Vymazal, 2011; Yan and Xu, 2014). Constructed wetlands are also an attractive alternative for wastewater treatment in developing countries, particularly in China where thousands of Constructed wetlands (CWs) have been functional as wastewater treatment technologies (Chen *et al.,* 2014).

- **Cost-benefit analysis**

As described in Brutland commisiion, cost-benefit analysis is considered as vital and mandatory evaluation procedure to be followed to achieve sustainable development of the ecological activities. It basically involves operation and investment costs, land acquisition, ecological benefits and energy consumption. Previous studies have described advantages of constructed wetlands over other conventional wastewater treatment technologies in terms of operation and maintenance costs and energy consumptions (Zhang *et al.,* 2012; Wu *et al.,* 2014). However, land requirement for constructed wetlands are high, making it the most limiting factor in its broader applications in areas where population density is high and land resources are scarce. Further additional innovations such as artificial aerations to enhance the removal performance, increase the life cycle cost of constructed wetlands.

- **Operation and design**

The criteria for constructed wetland operation and design include plant selection, site selection, wastewater type, substrate selection, plant material selection, hydraulic retention time (HRT), operation mood and maintenance procedures and water depth (Akratos *et al.,* 2009; Kadlec and Wallace, 2009). Particularly, the factors such as plant selection, water depth, substrate selection, hydraulic retention time (HRT), and feeding mood are crucial to create a feasible CW system and attain the sustainable performance.

- **Plant selection criteria**

Macrophytes which have unique properties relevant to the treatment process could play a deliberate role in CWs, and are considered to be the vital constituent of the design and operation of Constructed wetland treatments. However, only a few plant species have been largely used in this treatment system (Vymazal, 2013; Wu *et al.,* 2015). For the selection of macrophytes factors which are mainly are considered include:

- Tolerance of hyper-eutrophic and waterlogged-anoxic conditions
- Efficiency of pollutant absorption

Macrophytes mostly used in CW treatments include emergent plants, floating leaved plants, submerged plant and free-floating plants. More than 150 macrophyte species have been used in CWs globally, but only a limited number of these plant species are planted in CWs practically (Vymazal, 2013). The most commonly used growing species are *Typha* spp., *Phragmites* spp., *Scirpus* spp., *Iris* spp., *Juncus* spp. and *Eleocharis* spp. The most frequently used submerged plants are *Hydrilla verticillata*, *Ceratophyllum demersum*, *Vallisneria natans*, *Myriophyllum verticillatum* and *Potamogeton crispus*. The floating leafy plants include *Marsilea quadrifolia*, *Nymphaea tetragona* and *Trapa bispinosa*. The free-floating plants are *Eichhornia crassipes*, *Pistia stratiotes*, *Hydrocharis dubia* ,*Centella asiatica*, *Salvinia natans*, and *Lemna minor*.

- **Pollutant removal efficiency**

A study was conducted to evaluate the performance efficiency of constructed wetland system Vertical flow constructed wetlands were developed to treat domestic wastewater. During the experiment under plant impact, percentages of NH_4^+-N, COD, NO_3^--N and Total nitrogen removed in the system were up to 91.8, 85.4, 97 and 92.3 % respectively. Temperature sustainability were observed to have a great influence on the performance efficiency. Higher COD removal percentages were observed high temperature (19.8°C and 25.5°C) At 8.9°C, a sharp decline in COD removal percentage was observed. Temperature also impacted the NO_3^--N removal, biomass and soil microbial activity. CWs in the experiment had consistently achieved high removal efficiency (above 80%) for COD, TP and TN in all experiments (Zhou *et al.,* 2017).

A research conducted by Victor and his coworkers in 2016 elucidated the phytoremediation potential of *Pistia stratiotes* (water lettuce) on the elimination of domestic wastewater toxicity. Phytoremediation reduced, 50.04% of PO_4^{-3}, 58.87% of ammonium content, 82.45% of COD and 84.91% of BOD. After experimental period, metal contents in treated wastewaters decreased from 3.51 to 93.51% for water lettuce tanks (Victor *et al.*, 2016).

A study conducted in china on parallel horizontal subsurface flow planted with three plant species (*Phragmites australis, Typha latifolia* and *Scirpus planiculmis*). The plant removal efficiencies under saline condition were assessed. The major parameters measured in the effluent were chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen (TN), and total phosphorus (TP). The increasing order of metal accumulations in CWs was K>Ca>Na>Mg>Zn>Cu. More than 80% of metals were concentrated in the root part of *Pistia australis*. *Typha latifolia* had the best performance of pollutant removal, with average removal of 49.96 and 39.45% for COD and TN respectively. The effluent water quality is in accordance with the water standards set by China. (Xu *et al.*, 2017)

2.1.2.2 Biomechanical processes (Membrane bioreactors)

Membrane bioreactors are those technologies that offer biological treatment along with membrane separation processes. This term is more appropriately used for processes which involves the coupling of these two elements rather than the use of individual membrane separation technique. Treatment of domestic wastewater through conventional wastewater treatment techniques are usually carried out through a three-stage process: sedimentation followed by aerobic degradation of the organic matter and then another sedimentation process that remove biomass from the sewage waste. MBR technology involves elimination of two phase separation processes by filtration of biomass through the membrane. Water quality of the product is far higher than that produced by conventional treatment, avoiding the need for a further tertiary decontamination process (Figure 2.2) (Judd, 2008).

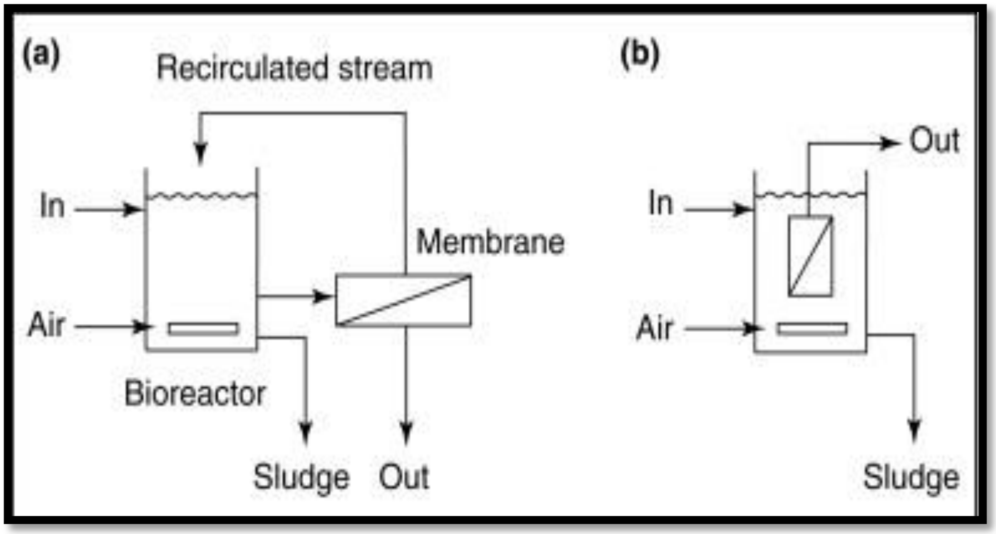


Figure 2.2: Configuration of membrane processes (a) Side stream membrane (b) Immersed membrane (Judd, 2008).

Studies have reported various advantages and disadvantages of MBR as mentioned below (Mutamim *et al.,* 2013; Zhang *et al.,* 2006):

Table 2.1: Advantages and disadvantages of membrane bioreactor technology

Advantages	Disadvantages
High-quality effluent	Higher energy costs
Lesser hydraulic retention times (HRT)	The need to control membrane fouling problems
Minimum sludge production	Potential high costs of periodic membrane replacement
Higher loading rates	
Potential for simultaneous nitrification and denitrification in long Solids Retention Time (SRT)	
Elimination of need for secondary clarifiers.	

- **Brief history**

The prior versions of MBR technology was introduced in early 1960s and was composed of a separate membrane filtration unit that was typically operated in cross-flow manner and was fed from an aeration tank. This arrangement, which is still in use for some applications, requires optimization of each process (Solids separation and biological treatment) but it requires a lot of energy to maintain sufficient velocity to control membrane fouling. Since mid-1980s a new concept of submergence of membrane in the aeration tank was introduces which have greatly reduced energy requirements and also membrane fouling.

Some of largest MBR plants manufacturers include Kubota (Japan), Zenon (Canada) leads with respect to installed capacity than Kubota, Kaarst, Germany (48,000 m^3/d in 2005), tertiary treatment at Qinghe, Beijing (400,000 m^3/d in 2011) and King County, WA, USA (136,000 m^3/d in 2011) and. Despite these developments, Membrane bioreactor is still a new technology with limited operational and design experience when linked with more than a century of activated sludge (Buer and Cumin, 2010).

- **Pollutant removal efficiency**

High strength wastewaters can be successfully treated by MBR which have typically very high concentrations of COD and BOD. By monitoring and maintaining parameters such as HRT, SRT, TMP, MLSS and Flux to an optimum condition, the finest performance of MBR can be achieved and also membrane fouling can be controlled. For wastewater with very high loading, it has to be treated before entering MBR to avoid membrane fouling. Some physiological characteristics (EPS, SMP, organic and inorganic matters and MLSS concentration) are difficult to control and result membrane fouling. However, some methods are there to reduce the fouling problems and enhance the performance of MBR to yield high quality effluents (Mutamim *et al.*, 2013).

A study was conducted to check the performance efficiency of pilot scale MBR system that was operated continuously for 485 days, treated domestic wastewater with temperature maintained between 8 and 30° C and hydraulic retention time was maintained between 4.6 to 8 hours. During the winter season, average removal efficiency of COD and BOD was 81 and 85% respectively at 8-15° C. However, in summers under more acclimatized conditions the removal efficiency of COD and BOD was 94 and 98% respectively and the average effluent COD value was not higher than 24 mg/l and BOD value not higher than 9 mg/l. Operational energy requirements were about 23 kWh/m^3. Biosolid that was produced during operation in all seasons was in the range of 0.051 g volatile suspended solids per gram COD removal (Shin *et al.*, 2014).

Another study was conducted to check the performance of MBR system to treat municipal wastewater at low temperature. Firstly, the impact of low temperature on membrane fouling was evaluated by continuously monitoring the trans membrane pressure and highest membrane fouling was observed at temperature below 10° C with a drop in membrane permeability by 75 %, thus higher deterioration of membrane was observed at lower temperature. With regard to the pollution control, highest removal of pathogen and micro pollutants were noted. The mean log reductions of 1.82, 1.94 and 3.02 log units were attained for norovirus GI, adenoviruses and norovirus GII respectively. Regarding trace metals, an average removal of > 80% was achieved for Cd, Pb, and V. The removal efficiencies with respect to trace metals were in the range of 30 and 60% (Gurung *et al.*, 2017).

2.2 Water quality parameters

Water quality is related to the chemical, physical, biological and radiological properties of water. Water quality monitoring is an important part of ecosystem analysis Chemical parameters include pH, Dissolved oxygen (DO), Total suspended solids (TSS), Chemical oxygen demand (COD) while turbidity and temperature comes under the category of physical parameters of water. Bacteria, algae and phytoplankton are the biological indicators of water quality (Hellawell, 2012).

Constructed wetlands improve the water quality in a number of ways to make it reusable. Processes involve in removing pollutants and improving water quality are settling of suspended matter, chemical precipitation and filtration through interaction of water with substrate, chemical conversion, adsorption and ion exchange on the surfaces of plants, substrate, sediment, and litter, uptake and transformation of nutrients by microorganisms and plants, natural die-off and predation of pathogens (Cooper & Findlater, 2013). Several studies have reported that because of high pollutant removal efficiency, low cost, easy operation and maintenance, and nutrient reuse, water quality improvement, Constructed wetlands have been documented as a suitable and effective wastewater management option for developing countries (Zhang *et al.,* 2015; Zhang *et al.,* 2012; Kadlec and Wallace, 2009; Stottmeister *et al.,* 2003). Furthermore, the removal of wastewater treatment parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), fecal indicator bacteria and pathogens, heavy metals and nutrients by constructed wetlands (CWs) are documented by various researchers (Tanaka *et al.,* 2006; Dan *et al.,* 2011).

Processes involved in improving water quality in MBR system are filtration and clarification through microfiltration or loose ultrafiltration membranes which is capable of removing suspended solids and microorganisms at higher mixed liquor suspended solids concentrations (Xagoraraki *et al.,* 2014; Chae *et al.,* 2006). Among the diverse treatment techniques, MBR technology has been considered as an attractive process for wastewater treatment by many scientists over the last few years. In comparison with conventional wastewater treatments, the MBR technology was reported as an efficient technology able to achieve satisfactory exclusion efficiencies of surfactants, organic substances and microbial contaminations. In other words, MBR technology has proved to be the most useful method for domestic wastewater treatment and improving water quality through physical along with biological treatment of wastewater. (Merz *et al.,* 2007; Lesjean and Gnirss, 2006; Liu *et al.,* 2005; Oschmann *et al.,* 2005). Study conducted by Hülsen and his coworkers evaluated the performance efficiency of MBR system in terms of water quality improvement and have reported strong potential of MBR technique for COD removal (<50 mg/L in the effluent), total phosphorus removal (<1 mg/L in the final effluent) and TSS removal (Hülsen *et al.,* 2016).

2.2.1 pH

pH is a numeric scale used to measure the acidity or basicity of an aqueous solution. It is assessed on basis of a set scale which range from 0-14. pH value of 7 is classified as neutral, 0-6 as acidic while from 8-14 is considered as neutral. pH is an important parameter involved in biodegradation of organic substances and also affects the solubility and toxicity of chemicals. 6.5-9 is the preferred pH range for the water to be reused (Jianquan & L.uhui, 2011).

2.2.2 Total Dissolved Solids (TDS)

It contains minor quantity of organic matter and inorganic salts dissolved in water. The major constituents are usually the cations magnesium, calcium and sodium, and the anions bicarbonates, carbonates, nitrates, sulphates and chlorides.

2.2.3 Turbidity

It is a measure of suspended particles in water and the particles include silt, clay, is a measurement of particles of matter suspended in water. These particles can be clay, silt, finely divided organic and matter, plankton and other microorganisms. It is not a measure of particles themselves rather it refers to the measurement of how light scatters when it is bounced by suspended fine particles in water. The target for the treated water turbidity is 0.1 NTU. It is a secondary indicator of total suspended solids. It is used to measure the efficiency of some disinfection procedures such as chlorination or ultraviolet light that needs straight exposure to the targeted contaminant (de Jonge *et al.*, 2014).

2.2.4 Total Suspended Solids (TSS)

These are the solids that remain in suspension form and it is contrary to the settling of matter. It lowers down the turbidity of water and raises the temperature of water. It causes hindrances in the path of sunlight being supplied to plants, hence result in lower DO concentration of the water (Verma *et al.*, 2013).

2.2.5 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is an important index for measuring organic pollution in water and is used in some states as a national standard to examine the aqueous organic pollution. The conventional process to measure COD is the closed reflux titration method by using sulphuric acid and dichromate reagent. Hence, COD can be described as the number of oxygen equivalents needed to oxidize organic pollutants in water (Latif and Dickert, 2015).

2.2.6 Biological Oxygen Demand (COD)

Biochemical Oxygen Demand (BOD) was first selected in 1908 by the U.K. Royal Commission on River Pollution as an indicator of the organic contamination of rivers. It is the ratio of amount of oxygen to the volume of system required by microorganism for their respiratory activity to degrade organic compounds when incubated at 20° C for 5 days. In other words, it is measure of organic pollution of water that can be degraded through biological means (Jouanneau *et al.*, 2014).

2.2.7 Coliforms

Coliform are the group of bacteria presents in polluted water and is found in feaces, vegetation and soil and act as an indicator of microbial quality of water. It comprises the group of enteric bacteria which are mainly gram negative, are rod shape and act as lactose fermenters. The coliform group comprises of *E. coli* but *Klebsiella* are also lactose fermenters at specified temperature therefore, considered as coliforms. They are responsible for a number of diseases in humans and animals (Feng *et al.*, 2014).

2.3 Relatedness of meteorological parameters with hydrological parameters

Impacts of meteorological on water quality parameters were documented in various studies and significant impacts and correlations were identified. Zhang and his coworkers have worked to explore the correlation among various physicochemical parameters (suspended solids and total dissolved solids) with meteorological parameters in a reservoir in China. A regression method, was used to examine the correlations among eleven physicochemical parameters and three meteorological factors (rainfall, wind speed and solar radiation). They concluded that the three meteorological parameters were positively correlated with suspended solids. Moreover, significant correlations between many water quality factors such as COD, BOD, TDS, total nitrogen, total phosphorus and EC and meteorological factors were exhibited. In the meantime, significant positive correlations between SS and meteorological factors were noted, which indicate that meteorological factors had impacts on physicochemical parameters dynamics (Zhang *et al*., 2017).

A study was conducted in Poland to check the impacts of weather conditions on the physicochemical composition of surface water of kettle ponds and found notable impacts of weather parameters on the ionic concentration of water of two hydrologically and geographically distinct regions. The variation was attributed to the vertical exchange of air due to influence of various meteorological factors (Major and Cieśliński, 2017).

Another study was conducted to check the relatedness of hydro-meteorological parameters and cyanobacteria bloom. Hydro meteorological parameters under study were temperature, precipitation and relative humidity while the water quality parameters include NH_3-N, COD, TP and TN. Results revealed the proliferation of cyanobacteria due to alterations in water quality conditions and hydro-meteorological factors. Correlation analyses have showed that the expansion of cyanobacterial concentration and chlorophyll density was sensitive to temperature differences. Moreover, because of irregular rainfall patterns have showed negative impact on cyanobacterial growth. Results of the study have recommended that strict policy application can resolve the water quality problems in eutrophic lakes (Baig *et al.*, 2017).

2.4 Application of microbes in the treatment systems

2.4.1 Phytoremediation system

In constructed wetland system although filtration is considered as significant process in the removal phenomenon, some additional interactions take place among plants, media and water. Many processes between them take place which are microbial processes, volatilization, sedimentation, chemical networks, sorption, photodegradation etc. The significance of microbial processes was further studied by many researcher as most of the reactions occurring in phytoremediation system are microbiologically mediated (Stottmeister *et al.*, 2003; Kadlec and Wallace, 2009). The most notable microbiota in wetland systems is present in the biofilm related to the plant's roots or attached to the filter bed material. This microbial community that is mainly created by interactions with water, is responsible for the pollutant degradation efficiency of the system. Role of microbes in denitrification process is well documented in various studies. The diversity of microorganisms involved in nitrification process in the constructed wetland system include aerobic denitrifying bacteria, denitrifying fungi and heterotrophic denitrifying microorganisms (Sleytr *et al.,* 2009). Furthermore, the variety of microorganisms in this system is critical for its consistent performance and functioning (Ibekwe *et al.*, 2003).

A study was conducted by Oopkaup and his coworkers in 2016 to identify the bacterial dynamics and structure of horizontal subsurface flow constructed wetlands (HSSF) by using sequencing and PCR amplification. Dynamics of bacterial community abundance and structure of a newly established horizontal subsurface flow (HSSF) pilot-scale wetland were studied using high-throughput sequencing and quantitative polymerase chain reaction methods. The most prominent phylum identified were *Proteobacteria*, followed by *Bacteroidetes* and *Firmicutes* in wastewater. Bacterial community of CWs was increased over time and was positively associated to the wastewater treatment proficiency. Higher diversity of microbial community and profusion of denitrifiers were predictors of the removal efficiency of nitrate, ammonia and total organic carbon in HSSFCW. The results of the study identified two processes involved in nitrogen removal as depicted in Figure 2.3

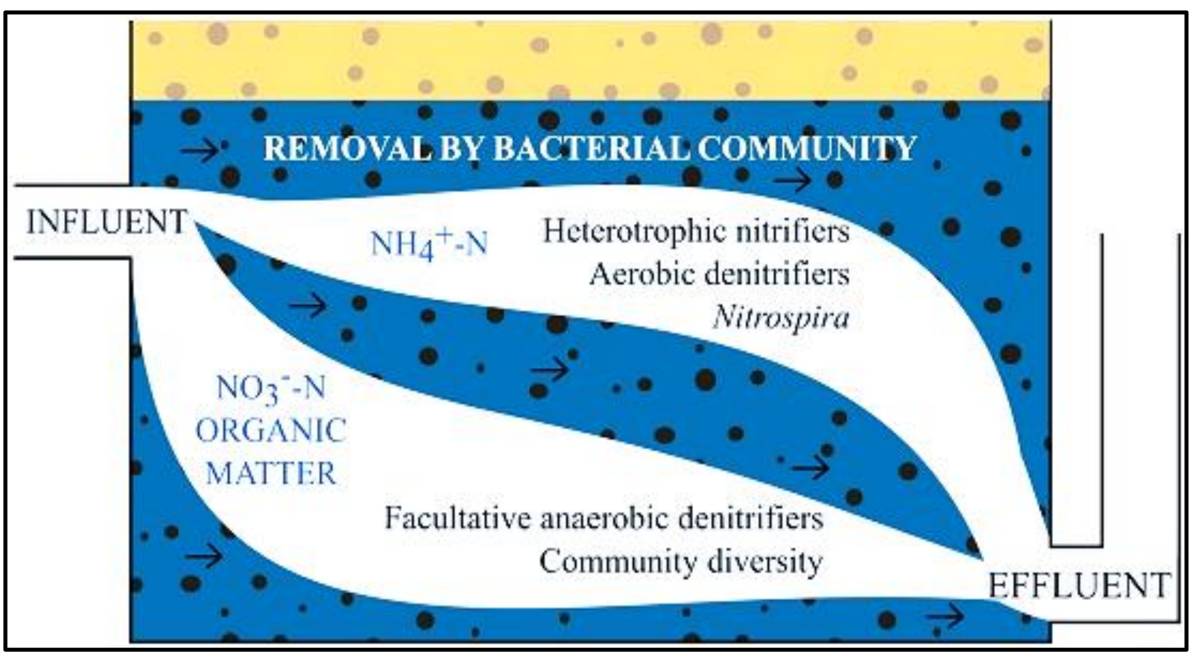


Figure 2.2: Bacterial processes involved in removal of organic matter and nitrogen compounds (Oopkaup et al., 2016)

2.4.2 MBR system

Incorporation of biological nutrient removal process with MBR has become an important process in the recent era for better performance of membrane and better effluent quality. Membrane bioreactor has been implemented successfully for simultaneous nitrogen, carbon and phosphorus removal from domestic wastewater. However, membrane bio-fouling is a major hindrance for the wide utilization of membrane because it results in significant decrease of permeate flux, it requires continuous replacement and cleaning of fouled membranes, and consequently, increases the operational cost of the process. Major factor involved in membrane fouling is sludge retention time (SRT). Various studies have reported that by increasing SRT, development of slow growing microorganisms can be enhanced as they are able to consume the macro-molecules as substrates and yield less biopolymers (Ahmed *et al.*, 2007, Choi *et al.*, 2017).

A study was conducted to check the variations in bacterial diversity and membrane fouling because of temperature. The results showed that density of microbial products and extracellular polymeric substances increased with decrease in temperature. Hence, resulted in membrane fouling because of increase in the transmembrane pressure. Previous studies have reported the abundance of proteobacteria. Furthermore, 16S rRNA sequencing results revealed *Nitrospira*, *Proteobacteria* and *Bacteriodetes* as the most dominant phyla. However, at lesser temperature, *α-proteobacteria Actinobacteria*, *Thiothrix* were relatively abundant. *Zoogloea* were found to be abundant at higher temperature. showed its presence (Ma *et al.*, 2013).

A study was conducted by Oh et al in 2017 to check the role of quorum quenching (QQ) bacteria in biofouling control. *Pantoea stewartii* was used as biofilm forming microorganism at lab scale. *Escherichia coli* strain was used as quorum sensing bacteria because it releases quorum sensing enzyme. The results revealed the strong potential of QQ bacteria in controlling biofilm formation by *P. stewartii*. QQ bacteria degraded the *N*-acylhomoserine lactone signals, which controls biofouling of membrane.

2.5 Biomass production from wetland macrophytes

Wetland macrophyte species have the potential of large biomass production. Biomass produced form it can be reutilized for various purposes as compost, feed for animals, fertilizers, paper industry, biodiesel and biogas productions. Potential for biofuel production from wetland species is neglected in spite of neutral CO balance and large biomass yield. Use of biomass as a fuel is restricted because as biomass contains elements such as K, Ca. Mg and Si which act as very reactive and have problematic behavior. However, by controlling temperature conditions, these elements can be vaporized. Therefore, study of thermal behavior is necessary to assess the suitability of wetland biomass to be used as fuel (Ciria *et al.*, 2005).

A study was conducted to investigate the biomass production of *Pistia stratiotes* and pollutant removal efficiency. Three different types of media were used water from the Sordo River (WSR), synthetic wastewater (SWW) and WSR modified with fertilizer. The experiment was performed during summers and winter. The productivity of plant was found higher in WSR and WSR + F during summers and spring. The study concluded that the phytoremediation system act as a dual purpose, high biomass production during summers and can effectively treat water from a polluted river (Robles-Pliego *et al.*, 2015).

The viability of using different constructed wetlands (CWs) plants for ethanol production was investigated in a study. Ethanol production from *Zantedeschia aethiopica Spreng*, *Iris pseudacorus Linn* and *Eichhornia crassipes* was examined. The maximum ethanol production was 8.27, 5.97 and 6.47 g per 100 g dry mass from *Iris pseudacorus Linn*, *Eichhornia crassipes*, *Zantedeschia aethiopica Spreng* respectively. Ethanol yield was 53.78%, 80.65%. and 90.78% respectively (He *et al.*, 2015).

Another study was conducted on constructed wetlands to check phosphorus and nitrogen uptake and the biomass potential of six perennial plants under study. Above ground biomass was harvested in replicates and nutrient content was measured. Results have indicated the highest biomass production by giant reed (Arundo donax) and it was about 5.59 kg/m^2 and its nutrient uptake was also maximum among canna, umbrella plant and alligator flag. Study concluded that giant reed and other wetland plants could be preferential macrophytes for bioenergy feedstock (Zhao *et al.*, 2017).

3 METHODOLOGY

3.1 Study Site

The wastewater treatment systems studied were the urban facilities used in NUST, H-12 sector Islamabad for the treatment of wastewater from residential apartments, academic blocks, schools, institutes and hostels. Wetland project was completed by the funding of UNESCO and is being maintained by NUST Research and development funds while funding agency for MBR plant was NUST R&D funds and Samsung Corporation Korea. The total population of NUST is around 6000 and it covers an area of 707 acres. The total volume of wastewater generated by NUST is about 200,000 US gallons per day and the flow into the treatment facility is maintained at 75000 US gallons per day at the inlet of CWs and about 7925 US gallons of wastewater is directed towards the inlet of MBR system. Layout of treatment is represented in Figure 3.1.

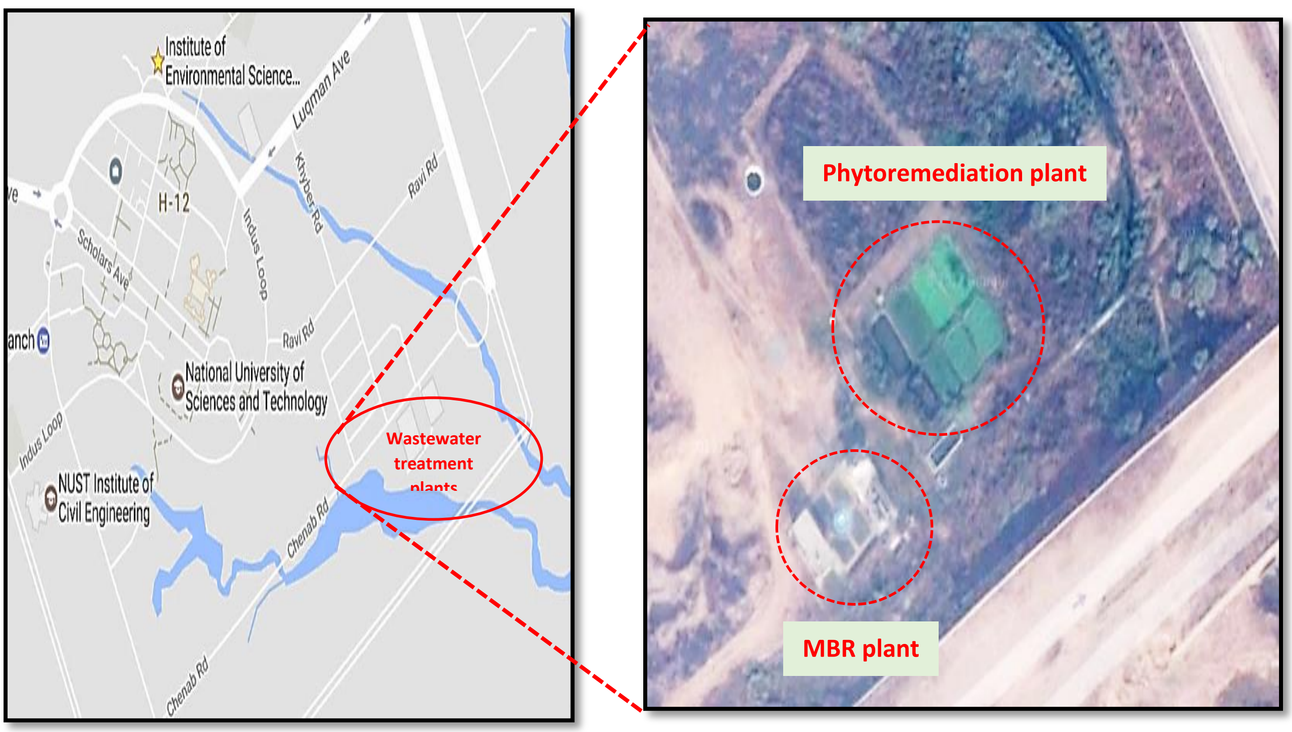


Figure 3.1: Layout of Phytoremediation and MBR plant

The layout of wetland system consists of sedimentation tank which is 35 feet long, 12 feet wide and 6 feet deep. CWs installed at NUST may treat around 0.1 Million gallons of water per day. About 18850 US gallons of wastewater is first pretreated in the sedimentation tank daily after that it is loaded in eight ponds sequentially having dimensions and characteristics mentioned in Table 3.1. Schematic layout of wetland setup is represented in Figure 3.2

Table 3.1: Description and characterization of vertical flow constructed wetlands

Pond No	Characteristics	Description	Dimensions (length, width, depth)	Total capacity (US gallons)
Inlet	Sedimentation tank	Sludge recovered to be used as fertilizer	35’ × 12’ × 6’	18850
Pond 1	*Planted with Typha latifolia*	Large persistent grasses native to tropical and temperate areas (Vymazal, 2011). Approx 15 plants per m^2 are cultivated	50’×22’×7’	41142
Pond 2	*Planted with Pistia stratiotes*	Light greenish-yellow shell like plant, long unbranched roots and is frost sensitive (Pott & Pott, 2002). Approx 10 plants per m^2 are cultivated	50’ ×22’ ×7’	57600
Pond 3	*Planted with Centella asiatica*	Considered effective for pollutant removal in summer however the removal potential can drop to even 50% in winters (Li. *et al.*, 2018). Approx 20 plants per m^2 are cultivated	50’ ×22’ ×7’	57600
Pond 4	*Planted with Centella asiatica*	Approx 20 plants per m^2 are cultivated	50’ ×22’ ×7’	57600
Pond 5	No plant specie planted	Only aquatic and sediment microbial community and natural settling are the removal mechanisms present	50’ ×22’ ×7’	57600
Pond 6	No plant specie planted	Only aquatic and sediment microbial community and natural settling are the removal mechanisms present	50’ ×22’ ×7’	57600
Pond 7	*Planted with Pistia stratiotes*	Approx 10 plants per m^2 are cultivated	50’ ×22’ ×7’	57600
Pond 8	Aeration pond	Aerators were installed to boost up oxygen level in the system	50’ ×22’ ×7’	57600
Outlet	Storage tank	Final treated water ready to be used for horticultural purposes	50’ ×22’ ×7’	

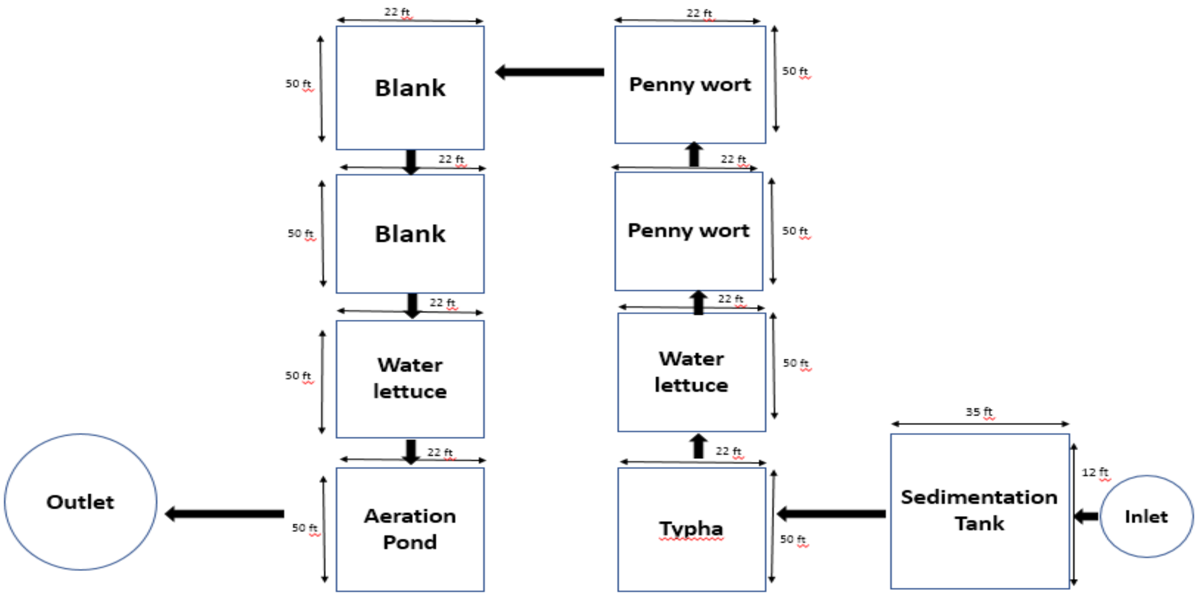


Figure 3.2: Pilot scale setup of vertical flow constructed wetlands. Arrows specify the flow of water

Whereas, NUST MBR plant comprised of a primary clarifier, five bio tanks and a membrane tank having total capacities of 1500, 12000 and 2350 liters respectively. The surface area of membrane is about 94.8 m^2. The system has the capacity of treating 12680 US gallons of water per day at 20 LMH while it may treat 6340 US gallons of water per day at 10 LMH. Figure 3.3 shows the schematic diagram of MBR plant.

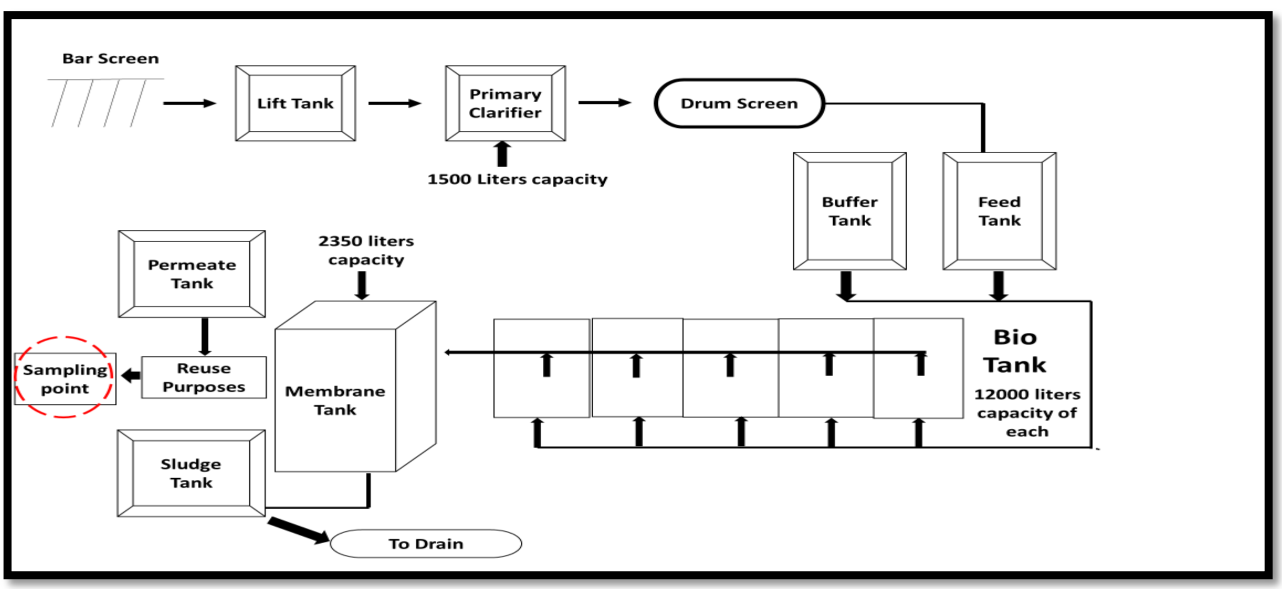


Figure 3.3: Schematic diagram of MBR plant (Hasnain, 2016)

3.2 Sampling

Samples were collected in glass sterile bottles that were first properly washed with detergent and were further rinsed with distilled water. After that they were autocalved at 120° C for 15 minutes and were dried in oven at 106° C for 2 hours. For physicochemical analysis samples were collected thrice in a month from inlet and outlets of Phytoremedaition system and MBR system from August 2016 to January 2017. The collected samples were immediately transferred to Environmental chemistry laboratory of Institute of Environmental Sciences and Engineering (IESE) for further physico-chemical and mirobial analysis. All the sampling and analysis processes were carried out under the standard method for examination of water and wastewater (APHA, 2017).

3.3 Analysis of water quality parameters

3.3.1 Physico-chemical parameters

Physico-chemical parameters analyzed in the study were temperature, pH , electrical conductivity, dissolved oxygen, turbidity, total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), chemical oxygen demand (COD), biological oxygen demand (BOD)

Table 3.2: Characterizes the parameters along with the instrument and method used for analysis.

Table 2.2: Characterization of physico-chemical and biological parameters

Parameters	Units	Instruments used	Method of analysis*
pH	-	pH meter (HACH 156)	Potentiometric method
Temperature	˚C	HACH session 1	Laboratory method
Conductivity	(µS/cm)	Conductivity meter (Ino Lab 720)	Potentiometric method
Total Dissolved Solids	(mg/L)	Conductivity meter (Ino Lab 720)	Potentiometric method
Total Suspended Solids	(mg/L)	Analytical Mass Balance	Gravimetric dried method
Dissolved Oxygen	(mg/L)	DO meter (HACH 156)	Potentiometric method

Turbidity	(NTU)	Turbidity meter (HACH 2100 P)	Laboratory method
Chemical oxygen demand	(mg/L)	Through titration	Closed reflux method
Biological oxygen demand	(mg/L)	DO meter (HACH 156)	Dilution method

*Standard method for examination of water and wastewater (APHA, 2017)

3.3.2 Microbiological parameters

Inlet and outlets samples were analyzed for total coliform(TC) that are indicator organisms for determining the coliform removal efficiency by Membrane Filtration technique according to the standard protocol (APHA, 2017) by using specific media (EMB agar media) and the measuring unit was CFU/100mL. Coliform removal efficiency was calculated by the formula:

Total coliform removal = $-Iog\left(\frac{C_o}{C_i}\right)$ (Hai *et al.*, 2014)

Where C_o and C_i are the outlet and inlet concentrations respectively

EMB agar plate preparation

Glass petri plates were autoclaved at 121°C for 15 minutes and were oven dried at 106°C for 2 hours. Eosin Methylene Blue (EMB) agar media was prepared by weighing 2.8 gms of media in 100 mL of distilled water in volumetric flask sealed with aluminium foil and were autoclaved. Molten liquid media was poured in already sterilized petri plates in sterile environment. Plates were then allowed to solidify for 20 minutes and were placed in incubator for 24 hours to confirm sterility.

Membrane filtration

Grab samples were placed near the filtration assembly and were unsealed. Serial dilution was performed and serially diluted sample was allowed to pass through filter paper (0.45 µm size) fitted in filtration assembly. Each filter paper having coliform bacteria retained in it was placed onto already prepared EMB agar media plates and were incubated for 24 hours at 37°C. After incubation, colonies were counted in colony counter.

3.4 Acquisition of meterological data

Meterological data was acquired from US-Pak Center for Advanced Studies in Energy (US-CASE), NUST. Daily mean data of ambient temperature, relative humidity, wind speed, air pressure and Global horizontal irradiance were acquired from August 2016 to January 2017. Effects of individual meterological parameters on the physico-chemical and microbiological parameter of the systems were analyzed for six monthly period.

3.5 Elemental analysis of plants

Root and shoot samples of *Pistia stratiotes* (Water lettuce), *Centella asiatica* (Penny wort) and *Typha latifolia* (Typha) were collected in the months of August and December 2016 and were transferred to Environmental Microbiology Laboratory at IESE, samples were washed thoroughly with distilled water to remove superficial dust followed by oven drying at 70° C for 24 hours. To reduce the particle size and achieve homogenity, samples were crushed in pestle and mortar in the crushing time for 3-5 minutes. Once the homogenity was achieved samples were stored in polypropylene flasks until further analysis. Before analysis, 2 gms of powdered sample was weighed and pressed at 20 tonnes/cm^2 for 60 seconds to obtain a cylinderical pellet having diameter of 20-40 mm. After that each pellet was put in a sample holder and placed directly in the X-ray beam of XRF elemental analyzer (Model: JEOL JSX 3202 M) for elemental analysis (Marguí *et al*., 2007).

3.6 Isolation of bacteria

Samples were collected from surface and sediments of Pond 2 and 7 of phytoremediation system planted with *Pistia stratiotes* (Water lettuce) and activated sludge of MBR system. Process of sample collection, storage and isolation was performed according to the standard method. Samples were collected in sterile sampling bottles and transported to the laboratory. Surface disinfection was performed through 70% ethanol to maintain sterility. After that serial dilution was performed and appropriately serially diluted samples were plated onto already prepared nutrient agar plates which were then incubated at 37°C for 24 hours. After incubation, the pure cultures of most dominating strains were prepared through streaking and used for further analysis (Garcha *et al*., 2016). Total of 19 strains were obtained and were designated as KB1-KB19.

3.7 Identification of isolated bacterial strains

Bacterial species were identified and characterized through morphological, biochemical and molecular characteristics. The details of which are mentioned below:

3.7.1 Morphological characterization

Investigation of the structure and form of bacterial colonies is named as colony morphology and is often used as a first step in bacterial characterization. Bergey's Manual of Determinative Bacteriology (Bergey's manual) was used to analyze the bacterial colonies morphologically. Table 3.3 describes the commonly observed morphological features along with their description (Tortora *et al.,* 2004).

Table 3.3: Morphological characterization of bacteria

Sr #	Morphological feature	Description
1	Size	small, large, filamentous, punctiform
2	Color	white, off white, yellow, orange, pink, green
3	Elevation	convex, umbonate, raised, pulvinated, flat
4	Margin	curled, entire, lobate, undulate
5	Surface texture	dry, smooth, wrinkled
6	Opacity	opaque, transparent, translucent

3.7.2 Biochemical characterization

- **Gram staining**

  It is a technique specific to distinguish between gram positive and gram negative bacteria based on the differences in their cell wall characteristics. Cell wall of gram positive bacteria is made up of thick layer of peptidoglycan and is able to retain crystal violet strain which lead to the purple or blue appearance of cell wall after staining while cell wall of gram negative bacteria is composed of thin layer of peptidoglycan hence, it cannot retain crystal violet strain and appears pink in microscope after staining (Alfred, 2011).

Procedure was followed as described by (Fawole and Oso, 2004).

- **Motility test**

  This test was performed by following the procedure as described by (Olutiola *et al.*, 2000).

- **MacConkey agar test**

  MacConkey agar test was performed by following the procedure as described by (Olutiola *et al.*, 2000).

- **EMB agar test**

  This test was performed by following the procedure as described by APHA, 2017.

- **Catalase test**

  Catalase test was performed by following the procedure as described by (Cheesbrough, 2006).

- **Oxidase test**

  Oxidase test was carried out by following the procedure as described by (Cheesbrough, 2006).

3.7.3 Molecular characterization

Molecular method namely isolated DNA sequence analysis was employed to carry out the molecular identification of bacterial strains isolated from surface and sediments of Pond 2 and 7 planted with *Pistia stratiotes* (Water lettuce) and activated sludge of pilot scale MBR system. The followed method was basically the culture dependent method.

3.7.3.1 Primer sequences

PCR primers and sequences used in this study are mentioned in Table 3.4. The sequences were compared with the NCBI (National Center for Biotechnological Information) gene bank database by using BLAST function (http://www.ncbi.nlm.nih.gov).

Table 3.4: Selected primers for PCR amplifications

Primers	Sequence (5'-3')	Targeted genes	Reference
518F	CCAGCAGCCGCGGTAATACG	16S rRNA	Waheed *et al.*, 2013
800R	TACCAGGGTATCTAATCC	16S rRNA	

3.7.3.2 DNA extraction

Genomic bacterial DNA was extracted by using Invitrogen Pure link Genomic DNA Mini Kit by following manufacturers instruction (Cat no K1820-01, USA). The detailed procedure is described in Annexure I.

3.7.3.3 Agarose gel electrophoresis

Agarose gel electrophoresis was carried out to visualize the extracted DNA. 1% (w/v) agarose gel was prepared by adding 0.6 gms of agarose gel in 60 ml of 1X TBE buffer. Ethidium bromide solution (50µg/ml) was added as a staining agent. Electrophoresis was performed at 100 volts for 30 minutes. After that gel was observed by placing it under UV transilluminator.

3.7.3.4 PCR amplification

PCR was performed to amplify the extracted DNA. The reaction mixture was prepared (25 µl) having composition mentioned in Table 3.5

Table 3.5: Recipe of PCR reaction mixture

Reagents	Volume (µl)
Taq PCR master mix	25
DNA template	1
Primer F(10µM)	2
Primer R(10µM)	2
Nuclease free water (doubled distilled H_20)	20
Total volume	50

For the 16SrRNA gene detection , the PCR program includes 5 min at 95°C for template denaturation, and 40 cycles for template amplification consisting of three steps: 95°C for 1 min for DNA denaturation into single strand, 61°C for 1 min for primer to anneal to their complementary sequences on either side of the target sequence, 72°C for 1 min for extension of complementary DNA strand from each primer and final elongation at 72°C for 10 min for Taq DNA polymerase to synthesize any unextended strand left.

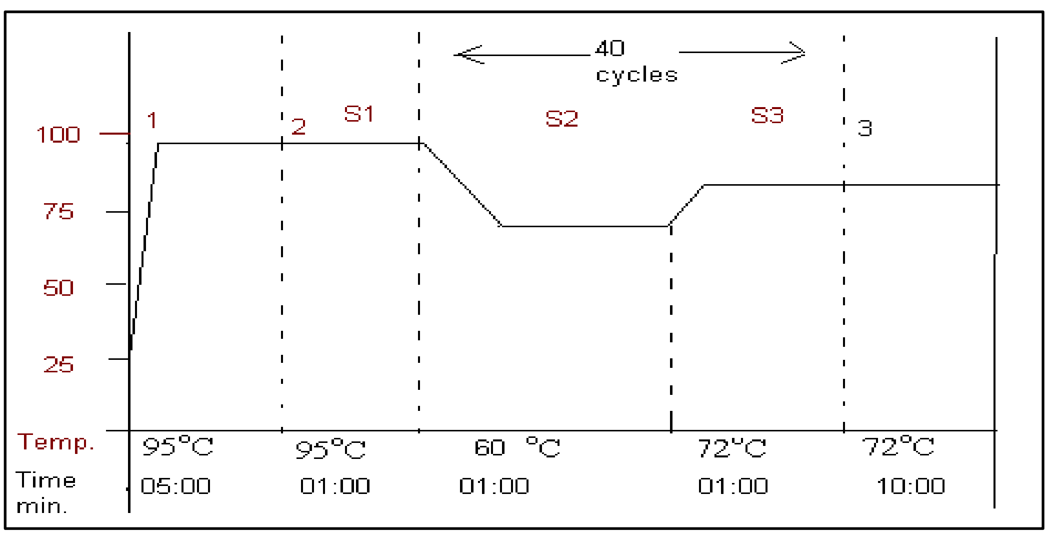


Figure 3.4: PCR program for 16SrRNA gene amplification

3.7.3.5 16S rRNA sequencing

PCR products were kept in ice box and the preserved isolates were sent to Genome analysis department Macrogen, Seoul , South Korea for 16S rRNA sequencing.

- **Phylogenetic analysis**

Phylogenetic analysis through phylogenetic tree show the evolutionary or ancestrol relationships among the various biological entities based on the differences in their genetic characteristics (Tamura *et al.,* 2013). Once the sequences were obtained, they were trimmed through Bioedit software and junk data was removed. Once the noise was removed and the sequences were properly trimmed they were analyzed through BLAST tool of National Center of Biotechnologcal Information (NCBI). After proper detection of the obtained species, accession numbers were obtained from NCBI gene bank library. FASTA sequences were run in MEGA 7 software to obtain the phylogenetic tree which showed linkages between the isolated strains and those at GENEBANK of NCBI.

3.8 Total biomass estimation

Sampling of predominant macrophyte specie was carried out in the months of August, September, November and December. Sampling species include *Pistia stratiote*, *Centella asiatica* and *Tpha latifola*. Samples were transferred to the laboratory after collection. Shoot, root lengths and fresh weights were measured. After that samples were wrapped in aluminium foil and were dried in oven at 70°C for 24 hours. Weight of dried samples were measured. Total biomass per pond was calculated by using following formula (Abe *et al.,* 2007).

$$\textbf{Total biomass production} = \textbf{No of plants per cubic feet} \times \textbf{Average plant production}\left(\frac{\textbf{kg}}{\textbf{cubic foot}}\right) \times \textbf{Total area of pond}(\textbf{cubic feet})$$

Relative growth rate (RGR%) of macrophyte species under analysis was measured by using following formula (Sastroutomo *et al.,* 1978).

$$\boldsymbol{RGR}\% = \frac{\ln \mathrm{W_t}}{\ln \mathrm{W_o}}\ \boldsymbol{x}\ \mathbf{100}$$

Where $\mathbf{W_o}$ is the initial weight while $\mathbf{W_t}$ is the weight after time **'t'**

3.9 Statistical Analysis

- **Standard deviation and mean calculation**

  Mean of replicate values was calculated and standard deviation was applied.

- **Correlation**

  Significant and non-significant effects of climatic parameters on physicochemical and biological parameters were noted with the level of significance set at $p<0.05$

- **T-test**

  Variations in the physicochemical and biological parameters with months were analyzed by applying t-test.

4. RESULTS AND DISCUSSION

4.1 Comparison of treated water quality of MBR and phytoremediation system

Inlets and outlets concentrations of the desired physicochemical and biological parameters of MBR (Membrane bioreactor) and phytoremediation system were measured thrice in a month from August 2016 to January 2017. Impact of climatic parameters on the physicochemical and biological parameters was observed and statistics was applied to analyze the significant and non-significant impacts. Monthly performance efficiencies of both Phytoremediation and MBR systems were analyzed and compared.

4.1.1 Phase I: Comparative analysis of climatic parameters effects on water quality parameters (physicochemical and biological) of treatment systems

Data of six meteorological parameters for six months were obtained from US-CASE, NUST. Parameters include ambient temperature, relative humidity, global horizontal irradiance, wind speed and air pressure. Effects of these parameters on water quality parameters is mentioned in detail below:

4.1.1.1 Monthly variations in water quality parameters with Global horizontal irradiance (W/m^2)

Global horizontal irradiance is the total amount of direct normal irradiation (DNI) and diffused horizontal irradiance (DIF). It is the sum of short wave radiation received by horizontal surface of earth (Lave *et al.*, 2015).

Negative correlation was noted between GHI and pH of influents and effluents of both systems ($r = -0.37$ for inlet, $r = -0.36$ for PS outlet and $r = -0.44$ for MBR outlet). Increase in the GHI resulted in better photosynthetic activity by plants which in turn resulted in more uptake of nutrients and lesser release of decaying organic matter, hence increase in GHI resulted in decrease in the value of pH for the phytoremediation system (Herrera, 2015). Same trend was observed for MBR system as depicted in Figure 4.1 (Annexure II).

Significant negative correlation of EC and GHI was noted for inlet and outlet samples of phytoremediation system (r = -0.99 for inlet and r = -0.90 for outlet) while slightly positive correlation of outlet of PS and DO and was observed (r = 0.59) because at lower radiations, less growth of plants hence lesser release of oxygen by the phytoremediation system overall. In case of MBR system a slight influence of GHI on EC were noted (r = -0.16) however slightly negative correlation was observed between GHI and DO (r = -0.64) as represented in Figure 4.1 (Annexure II). DO between 2 and 5 mg/L was maintained in the MBR via aeration using air compressor and diffuser system as compared to natural DO environment in the constructed wetland (CW) system. MBR is not significantly dependent upon ambient meteorological conditions as compared to CWs.

Significant negative correlation was observed between GHI and turbidity of influent and effluent samples of both systems (r = -0.87 for inlet, r = -0.75 for PS outlet and r = -0.67 for MBR outlet) while TSS of inlets showed significant positive correlation with GHI (r = -0.87). Outlets of both systems have showed non-significant correlation with both turbidity and TSS (Figure 4.1 Annexure II).

Turbidity and TSS shows the inverse relation in the phytoremediation system because TSS consists of minerals, clay, organic and inorganic matter which affect the transparency of water. While turbidity of water depends upon other factors like suspended particles, morphology of surface area which has an effect on the absorption and reflection of light (Mustapha *et al.*, 2013). Similar negative correlation inclinations were observed in water systems (Lagomarsino *et al.*, 2015).

Increase in GHI resulted in decrease in the concentrations of COD and BOD of inlet (r = -0.93 for COD and r = -0.6 for BOD). However, not significant impact was observed in the outlet concentrations of COD and BOD of both treatment systems (Figure 4.1 Annexure II).

Strong positive correlation was observed between GHI and total coliform concentration in the inlet (r = 0.92). However, concerning outlet of phytoremediation and MBR system non-significant impact was observed (r = 0.23 for PS outlet and r = 0.12 for MBR outlet).

4.1.1.2 Monthly variations in water quality parameters with Air pressure (hPa)

Barometric pressure affects the amount of gas that can dissolve in water. More gas, such as oxygen, can dissolve in water under higher barometric pressure than under lower barometric pressure. For instance, more oxygen is dissolved in water at sea level than at high altitudes as described by Henry's law (Sander, 2015).

Significant positive correlation was observed between air pressure and inlet-outlet value of pH of both systems (r = 0.73 for inlet, r = 0.62 for CWs outlet and r = 0.72 for MBR outlet). However, at highest air pressure in January, pH value got lower as depicted in Figure 4.2 (Annexure II).

Significant positive correlation of EC with air pressure was observed at inlet and outlet of phytoremediation system (r = 0.95 for inlet & r = 0.93 for PS outlet) as shown in figure 4.22(b). Slightly negative correlation of EC with air pressure was noted at outlet of MBR system (r = -0.2). Strong positive correlation was observed between air pressure and DO at inlet and outlet of MBR system (r = 0.90 for inlet & r = 0.72 for MBR outlet). However, negative correlation was noted between air pressure and DO of PS outlet (r = -0.6). This is represented in Figure 4.2 (Annexure II).

Significant positive correlation was observed between air pressure and turbidity of inlet and outlet of both treatment systems (r = 0.93 for inlet, r = 0.80 for PS outlet and r = 0.77 for MBR outlet) while significant negative correlation was observed between TSS concentrations of inlet and air pressure (r = -0.94) while in case of correlation with outlets; positive correlation was identified between air pressure and PS outlet (r = 0.66) and non-significant correlation was observed between air pressure and MBR outlet (r = -0.21) as represented in Figure 4.2 (Annexure II). Processes involved in improving water quality in MBR system are filtration and clarification through microfiltration or loose ultrafiltration membranes which is capable of removing suspended solids and microorganisms at higher mixed liquor suspended solids concentrations (Xagoraraki *et al.,* 2014; Chae *et al.,* 2007). Furthermore, the removal of wastewater treatment parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), fecal indicator bacteria and pathogens, heavy metals and nutrients by constructed wetlands (CWs) are documented by various researchers (Tanaka *et al.,* 2013; Dan *et al.,* 2011).

Positive correlation of COD and BOD with air pressure was noted. Increase in air pressure resulted in increase in the values of COD and BOD of inlet and outlet of MBR and Phyoremediation system.Strong positive correlation was observed between air pressure and COD-BOD of inlet (r = 0.96 for COD & r = 0.72 for BOD). COD-BOD concentrations in the outlets of phytoremediation system had slightly positive correlation with air pressure (r = 0.62 for COD & r = 0.67 for BOD) However, not much influence of air pressure on COD-BOD value of outlet of MBR system was observed (r = 0.3 for COD & r = 0 for BOD) as depicted in Figure 4.2 (Annexure II). Slightly negative corelation of air pressure and total coliform concentration was noted for inlet and phytoremedaition system (r = -0.49 for inlet & r = -0.55 for PS outlet) while regarding MBR system no correlation was noted between air pressure and total coliform concentrations as depicted in Figure 4.2 (Annexure II).

4.1.1.3 Monthly variations in water quality parameters with Wind speed (m/s)

Wind is the movement of air from higher pressure areas to lower pressure depending upon the fluctuations in air temperature (Wanninkhof, 2014). Lower wind speed increases residence time of pollutants and in turn result in lower Dissolved oxygen concentration, hence affect the photosynthesis process. Study conducted by Wood and Chang in 2006 reported another consequence of wind speed, as lower wind speed result in lower water pollutant dispersal and vice versa which overall effect pollutant removal efficiency of systems.

Increase in wind speed, correspondingly increases the values of pH of inlet and outlets of the operating systems for the whole six monthly period. Significant positive correlation of pH and wind speed was noted (r = 0.21 for inlet, r = 0.47 for PS outlet & r = 0.39 for MBR outlet) as represented in Figure 4.3 (Annexure II).

Slightly positive correlation was observed between windspeed and electrical conductivity of inlet (r = 0.009) and outlets (r = 0.0053 for PS outlet & r = 0.157 for MBR outlet) of both treatment systems (Figure 4.3 Annexure II). DO concentrations in the inlet had slight positive correlation with wind speed (r = 0.042) while outlet of phytoremediation system had negative correlation with the wind speed (r = -0.32). Outlet of MBR system had non-significant relation with wind speed as depicted in Figure 4.3 (Annexure II). This correlate with the study conducted by Kann and Welch in 2005 where lower wind speed affected the water column stability and positive correlation between wind speed and dissolved oxygen was observed. Lower wind speed in July had resulted in lower DO concentration in the system overall in July-August 2005.

Turbidity of inlet had slightly positive correlation with wind speed (r = 0.67) while turbidity in outlet of both systems had non-significant correlation with wind speed (Figure 4.3 Annexure II).). Regarding total suspended solids concentrations positive correlation was noted between TSS of inlet and wind speed (r = 0.43) while non-significant influence of wind speed on TSS concentrations of outlets was observed. This is perfectly in line with the study conducted by Zhang and his coworkers in 2017, where suspended solids (SS) were significantly positively correlated with wind speed and higher wind speed in spring correspondingly increased SS concentrations. Increase in the SS concentration may be attributed to the sediment resuspension as reported in the previous studies (Wu *et al.*, 2014). Wind would induce sediment resuspension, thus leading to the increase in SS concentration. However, despite of maximum windspeed in December, not much fluctuations in TSS concentrations were observed (Figure 4.3 Annexure II).

Increase in windspeed resulted in significant negative fluctuations in COD and BOD concentrations of outlets (r = -0.67 for Phytoremediation system outlet and r = -0.54 for MBR outlet) of both treatment systems under study and non-significant impact of RH(%) on inlet concentration was observed as depicted in Figure 4.3 (Annexure II). However, no impact of wind speed on BOD concentrations of outlets of MBR system was noted.

With decrease in wind speed, decrease in coliform concentration in the inlet and outlet of phytoremediation system was observed up to the month of November however in December, maximum wind speed resulted in lowest coliform concentrations in inlet and outlet of phytoremediation system. Concerning MBR system, not much impact of wind speed on total coliform concentration was noted (Figure 4.3 Annexure II).

4.1.1.4 Monthly Variations in Water Quality Parameters with Ambient Temperature (°C)

Temperature has a prominent impact on the pollutant removal efficiencies as well as total pollutant loads of both treatment systems as biomass, biodegradation and microbial community structure is extremely reliant on temperature fluctuations (Meng *et al.* 2014; Faulwetter *et al.* 2009).

pH varies from 7.07 to 7.765 throughout the experimental period. Highest pH was noted in December i.e. 7.765 while lowest were observed in September i.e. 7.07 as depicted in (Figure 4.4-Annexure II). pH of MBR outlet was found higher than inlet and outlet of phytoremediation system because in MBR system alkaline environment is created to buffer the hydrogen ion created in the nitrification process which in turn lead to increase in pH in the final effluent (Iorhemen *et al.* 2016). pH was highest in December i.e. 7.59, 7.64 and 7.65 for inlet, phytoremediation system outlet and MBR outlet. Negative correlation was observed between ambient temperature and pH of inlet (r = -0.63) and outlets (r = -0.56) for Phytoremediation system and r = -0.77 for MBR outlet of treatment systems as depicted in (Figure 4.4-Annexure II). pH of treated water was found within permissible limits set by EPA (6-10) to be fit for agricultural purposes.

Figure 4.4 in Annexure II indicates monthly variation in EC and DO concentrations in the influent and effluent samples. Not significant variations in EC were observed with months in both treatment systems. EC concentration in the treated water is found within the permissible limit of 3500 mg/L set by EPA to be fit for agricultural purposes.

The concentration of DO in the influent sample was very low (up to 0.08 mg/L in august) which is because of the higher microbial content which consume oxygen for degradation. An important improvement in the water quality of outlets was significant. Oxygen content in water sample of outlet of MBR was higher than the rest of samples. DO value of 5.21mg/L was observed at temperature as low as 18.25°C in MBR system. At higher temperature reduction in DO levels up to 0.08 mg/L was noted which indicates that warm water cannot endure DO in high concentrations. Significant negative correlation (r = -0.93) of EC with temperature was noted in case of phytoremediation system while positive correlation (r = 0.25) between EC and ambient temperature was observed for the MBR system. Negative correlation between DO and ambient temperature of inlet and MBR outlet was observed (r = -0.93) and positive correlation with

Phytoremediation outlet (r = 0.43) was identified. A study was conducted by Akratos & Tsihrintzis in 2009 to study the effects of temperature on DO of the system and have reported higher DO values in winters, when solubility of oxygen in water was higher and lower values were reported in summers because of its least solubility.

Significant negative correlation was observed between ambient temperature and turbidity of inlets (r = -0.95) while significant positive correlation (r = -0.98) was observed between TSS and ambient temperature of inlets. Not much significant variations of turbidity and TSS value of outlets of both systems with ambient temperature were observed. Increase in the ambient temperature resulted in increase in the temperature of water which enhanced the self-diffusion co-efficient of water. So, higher concentration of TSS at higher temperature can be attributed to this diffusion coefficient. The hollow-fiber (HF) membrane module used in MBR has a pore size of 0.03 µm resulting in consistently low turbidity and TSS and not dependent upon ambient temperature. This is in line with the study carried out by Ahsan and his coworkers in 2005.

Negative correlation was observed between ambient temperature and COD, BOD concentrations in the influent sample (r = -0.96 for COD & r = -0.63 for BOD) and effluents of Phytoremediation system i.e. r = 0.53. However, no correlation was noted between ambient temperature and concentrations of BOD in MBR effluent (Figure 4.4-Annexure II). Due to dual treatment of biological treatment followed by membrane filtration resulted in consistently low COD and negligible BOD in MBR and the ambient temperature may only influence the influent COD and BOD of raw water but not the effluent quality of MBR.

Studies have reported that the COD concentration in the system is because of the organic matter and the concentration of this organic matter is highly dependent on microbial activities of aerobic and anaerobic bacteria (Vymazal, 2009).

Positive correlation of ambient temperature and total coliform concentration was observed at the inlet (r = 0.60). Decrease in temperature resulted in increase in the total coliform concentration of phytoremediation system with the exception in winters where highest coliform concentration was noted. Coliform concentrations in the outlet of MBR system remain consistent and no significant impact of temperature on TC concentration of MBR outlet was observed due to membrane rejection of bacteria

4.1.1.5 Monthly variations in water quality parameters with Relative humidity (%)

Water vapor content is the total percentage of saturation vapor pressure of water at a given temperature. Various factors influence the water vapor content of atmosphere and these are air temperature, wind direction and also nearby water bodies have influential impact on water vapor content. With variations in the relative humidity, friction velocities in the water content will be altered and lower humidity decreases particle cohesion hence the total amount of pollutant holding capacity of water will be altered (Csavina et al. 2014).

Significant variations in pH of both setups were observed with the fluctuations in the relative humidity. Highest pH was observed in December for both setups when the relative humidity was least while the lowest pH was noted in September when the relative humidity was highest. Significant negative correlation was observed between RH (%) and pH of outlets of both setups (r = -0.94 for Phytoremediation and r = -0.87 for MBR). This whole trend is depicted in Figure 4.5-Annexure II. Positive correlation was observed between EC of inlet and relative humidity (r = -0.8). While negative correlation was noted between EC of outlets of both systems and relative humidity (r = -0.69 for CWs and r = -0.33 for MBR). While in case of DO positive correlation was observed between relative humidity and inlet and outlet of phytoremediation system. However, DO of outlet of MBR system showed the slight negative correlation with relative humidity (r = -0.49) as shown in Figure 4.5-Annexure II. Maximum value of dissolved oxygen in winter and increase in the concentration with relative humidity can be attributed to the prevailing wind conditions which permits the increase in the solubility of oxygen. This is in line with the previous studies (Fishar, 1999; Ali, 2008)

Significant increasing trend was observed between TSS of inlet and relative humidity (r = 0.64) while, relative humidity had not shown any significant impact on the TSS concentrations of outlets of both systems as depicted in Figure 4.5-Annexure II. Significant declining trend was noted between turbidity of inlet and relative humidity (r = -0.71) while non-significant correlation was noted between turbidity of outlets of both systems and relative humidity as depicted in Figure 4.5-Annexure II. With the increase in relative humidity decrease in COD and BOD concentrations of inlet were observed (r = -0.53 and r = -0.92) however, concerning outlets of both setups non-significant correlation existed between relative humidity and COD, BOD concentrations. Further the reported work of the previous studies conducted by Sankararajan and his coworkers in 2017 indicated the decrease in BOD concentration up to 5% with increase in relative humidity through genetic programming.

Strong positive correlation was exhibited between total coliform concentration of inlet and outlets of phytoremediation setup and relative humidity (r = 0.7) while concentrations of total coliform in the outlet of MBR setup were not influenced by relative humidity (Figure 4.5-Annexure II).

4.1.2 Phase II: Comparison of monthly performance efficiencies of systems

4.1.2.1 Turbidity removal efficiency

The average removal efficiency of Phytoremediation system was 90.1, 90.9 and 82.2% in August, October and December respectively. Concerning MBR system the removal efficiency in terms of percentage is as follows: 99.1, 99.8 and 98% for August, October and December respectively as represented in Figure 4.6.

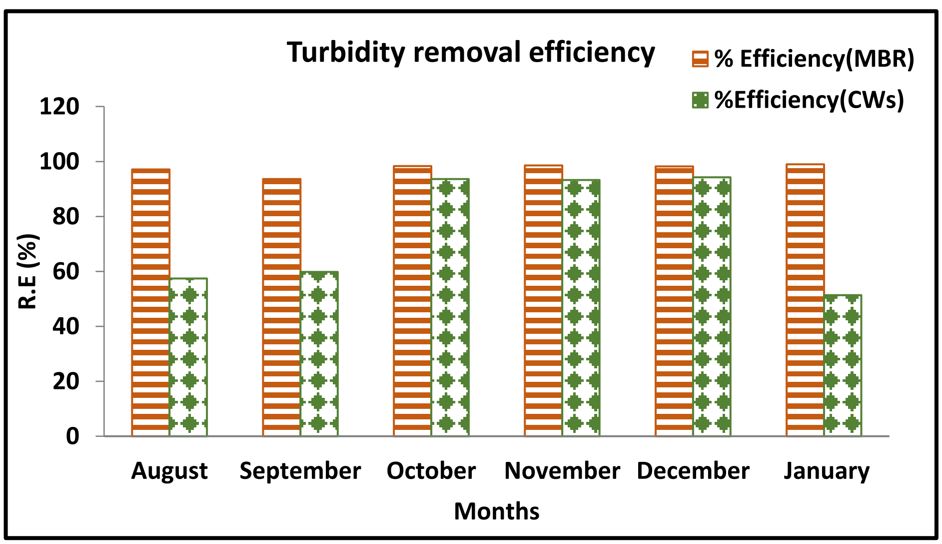


Figure 4.6: Monthly variations in turbidity removal efficiency of phytoremediation and MBR system

4.1.2.2 TSS removal efficiency

TSS removal efficiency of the wetland system was 90.8% in August thereafter a rapid reduction in TSS efficiency was observed in January i.e. 82.45%. This may be attributed to increase in organic matter because of death and decay of plants which in turn resulted in rise in suspended solids (Weerakoon, 2016). However, removal efficiency of MBR system remains consistent except only slight variations were observed. The increasing trend of removal efficiency of MBR system with seasons is as follows: 98.6%(Aug-Sep)>97.5%(Oct-Nov)>95.3%(Dec-Jan) (Figure 4.7). This is due to lesser survival of microbial MBR specie at lower temperature. This is in line with the study carried out by Chae & Shin in 2007 and they have reported similar results of about 98% TSS removal efficiency of MBR system at 30°C.

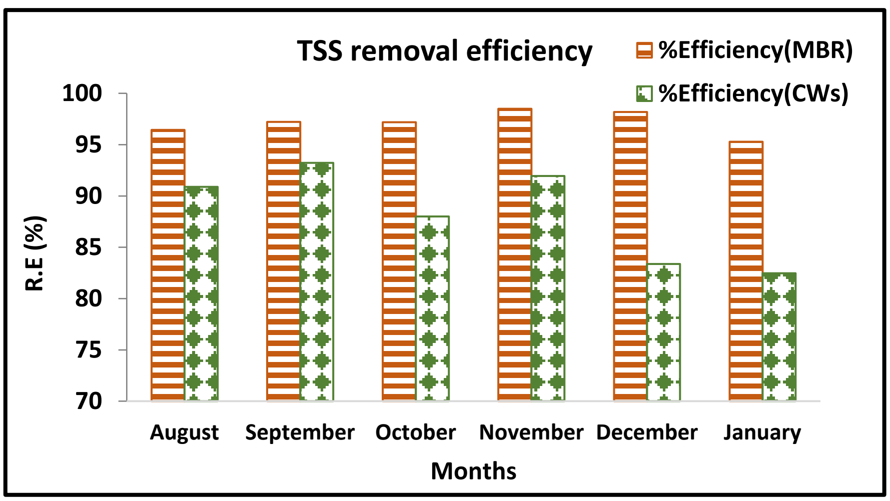


Figure 4.7: Monthly variations in TSS removal efficiency of phytoremediation and MBR system

4.1.2.3 COD removal efficiency

When removal efficiency was observed for each month three groups were noted (Aug-Sep), (Oct-Nov) and (Dec-Jan), with an average removal efficiency of 82.7, 78.84 and 76.6% respectively for MBR system while it was 67.7, 48.9 and 43.5% respectively for Phytoremediation system (Figure 4.8). Winter group showed the lowest efficiency for COD removal for both systems potentially due to decrease in temperature (Mancilla *et al*., 2013). The removal of pollutants increases with establishment and growth of plants (Rai *et al.*, 2013).

During winter season, plant density was decreased which in turn resulted in lower organic matter removal by phytoremediation system while in case of MBR system removal efficiency was slightly affected by the temperature changes and it was low in winters because of greater membrane fouling which is because of increased production of supernatant organics (Qu *et al.*, 2014). Greater than 80 % COD removal efficiency was reported by Choi and his coworkers in 2017 that was achieved in full scale MBR system and it is in line with the results reported in current study.

Favorable temperatures have high influence on microbial compositions and microbial growth rates. Enzymes of microbes are highly temperature dependent and greater membrane fouling occurred at lower temperature (Lin *et al.*, 2014; Liberman *et al.*, 2016).

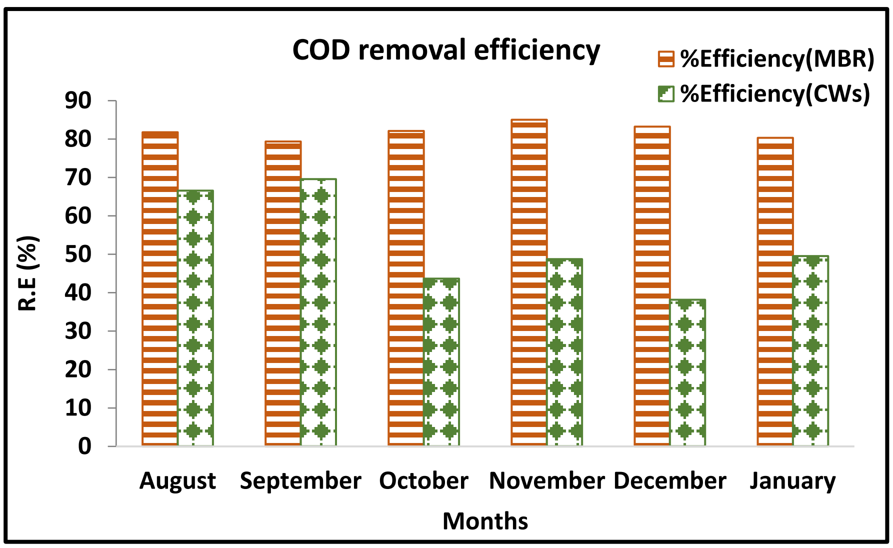


Figure 4.8: Monthly variations in COD removal efficiency of phytoremediation and MBR system

4.1.2.4 BOD removal efficiency

Removal efficiency in terms of percentage of the CWs was 67.6, 66.9 and 65.3% for (Aug-Sep), (Oct-Nov) and (Dec-Jan) respectively. In winters, due to death and decay of macrophytes in the CWs, BOD removal was slightly reduced but overall BOD removal depends on other factors too like sedimentation, absorption, microbial metabolism and biochemical activity in the system so overall significant variation in the removal efficiency was observed. This is in line with the study carried out by Weerakon and his coworkers and have reported increase in BOD removal by 1% in the planted system as compared to unplanted system (Weerakon *et al.*, 2016). Regarding MBR system 100% BOD removal efficiency was achieved for the entire period (Figure 4.9). A study was conducted by Garfi and his coworkers on the dependency of BOD removal by wetland system on temperature and have stated higher BOD removal in summers as compared to winters (Garfi *et al.*, 2012; Wang *et al.*, 2016). BOD of treated water from both systems is found within the permissible limits set by EPA that is 80 mg/L to be used for agricultural purposes.

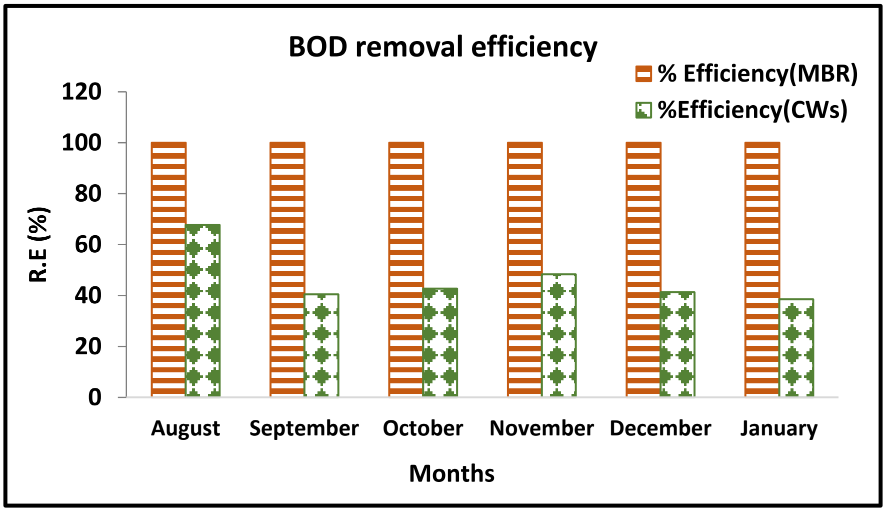


Figure 4.9: Monthly variations in BOD removal efficiency of phytoremediation and MBR system

4.1.2.5 Total coliform removal efficiency

Highest efficiency was achieved by phytoremediation system in September (88.9%) because of maximum growth of macrophytes and higher UV radiations that resulted in maximum coliform removal by the system while lowest efficiency was in January i.e. 56% (Figure 4.10). A study was conducted which reported decline in the coliform removal by 6% with the decrease in temperature as well as growth of plants (Weerakoon *et al.*, 2016).

Coliform removal by MBR system varied from 99.1 to 90% in September and January respectively. Removal efficiency remains constant except only slight variations with temperature as higher temperature stimulates the growth of bacteria associated with membrane (Liberman *et al.*, 2016). The finding showed that TC were the most reduced microbes and their concentrations were below the permissible limits set by EPA to be fit for agricultural purposes.

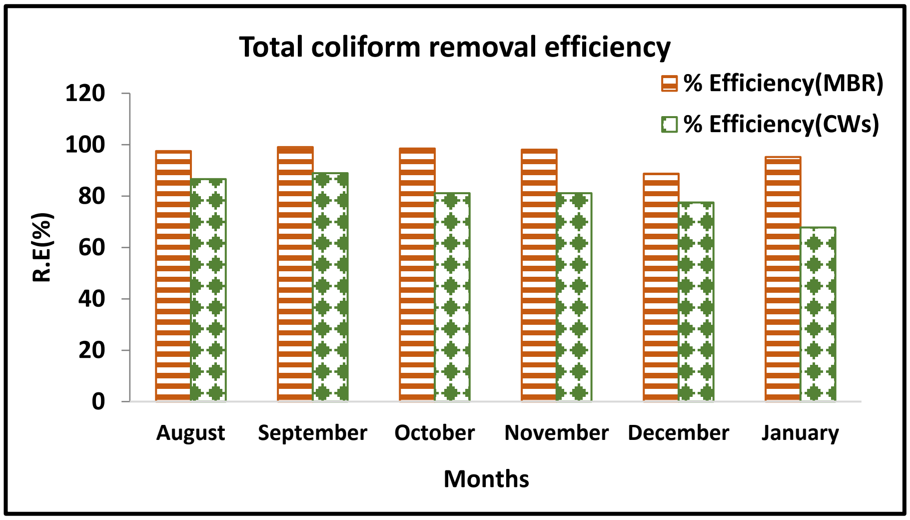
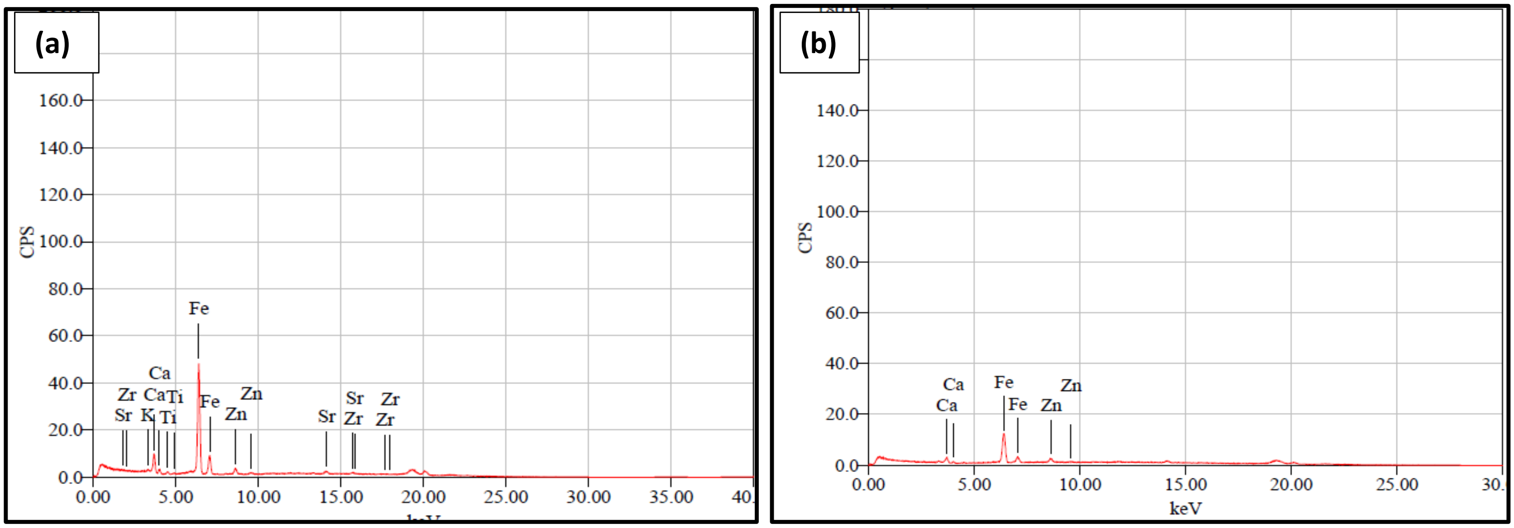


Figure 4.10: Monthly variations in BOD removal efficiency of phytoremediation and MBR system

4.1.3 Phase III: Elemental analysis of plants (XRF analysis)

XRF is a very beneficial analytical technique to study the chemical configuration of different materials. It is also one of the most common procedures for qualitative and quantitative estimation of elemental composition of all type of materials (Sarma& Goswami, 2016). The results obtained from elemental analysis of roots and shoots of three Phyto remediating plant species (*Typha latifolia*, *Pistia stratiotes* and *Centella asiatica*) are represented in below Figures (4.11-4.15). Roots of all plants had the higher percentages of different elements than shoots. Comparative analysis between different months have showed the highest uptake of elements in august as compared to December. This may be attributed to the decrease in the relative growth rate of all plants. Roots of *Typha latifolia* had the highest elemental uptake than the rest of samples while no significant variations in the uptake of elements by *Centella asiatica* in both months were observed because of the consistency in the relative growth rate. A similar study was conducted which showed the highest trace elemental uptake and removal by *Typha latifolia* and this was due to the least effects of these elements on the growth of *Typha latifolia* plant (Salem *et al.*, 2016). It also coincides with the study conducted by Atkinson and his co-workers in 2010 and identified reduction in relative growth rate of different wetland plants in different seasons and highest uptake was observed in roots of different wetland species.



(a)

Quantitative result

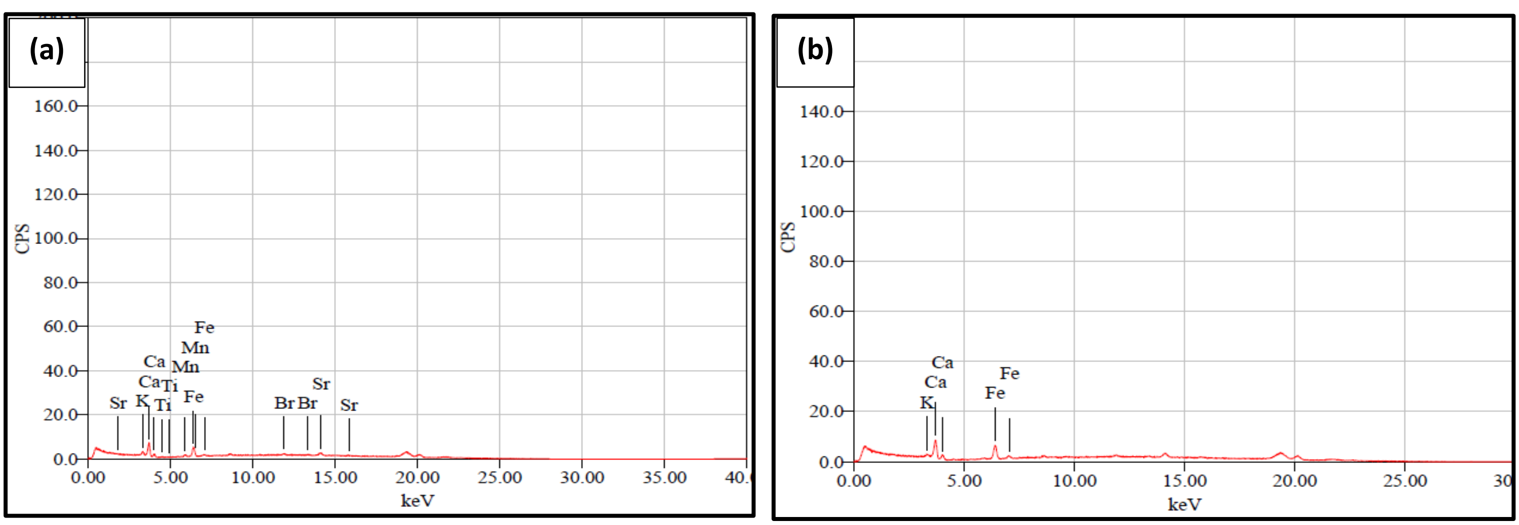
Element	ms%	mol%	Sigma	Intensity
19 K	3.5981	4.7176	0.3849	790
20 Ca	22.3499	28.5887	0.2213	8400
22 Ti	2.1487	2.2997	0.1100	1182
26 Fe	64.3304	59.0558	0.0782	59834
30 Zn	4.6689	3.6617	0.1190	3333
38 Sr	1.9226	1.1249	0.1496	1769
40 Zr	0.9815	0.5516	0.1806	937

(b)

Quantitative result

Element	ms%	mol%	Sigma	Intensity
20 Ca	22.7016	29.5300	0.5097	2340
26 Fe	64.8619	60.5513	0.1865	15986
30 Zn	12.4365	9.9187	0.3060	2182

Figure 4.11: Elemental analysis of Typha latifolia (roots) in (a) August and (b)December



(a)

Quantitative result

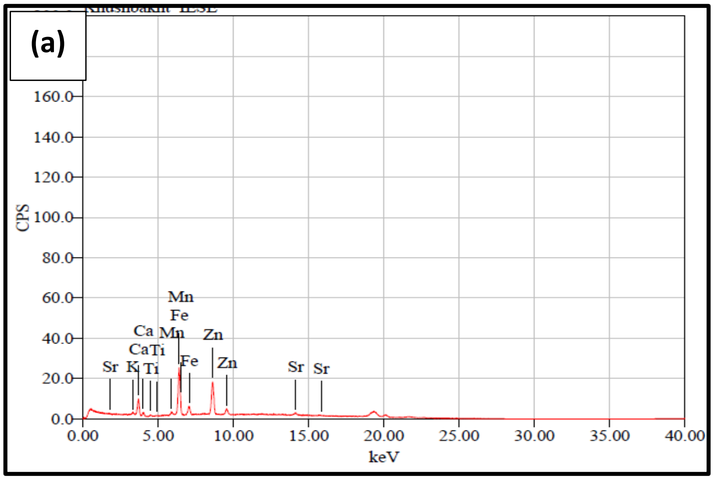
Element	ms%	mol%	Sigma	Intensity	K ratio
19 K	16.2339	18.1989	0.6727	1657	0.0017160
20 Ca	55.5151	60.7164	0.5671	6615	0.0040939
22 Ti	2.0176	1.8464	0.3748	265	0.0000980
25 Mn	4.1383	3.3020	0.2066	1077	0.0002691
26 Fe	16.8581	13.2321	0.1855	5369	0.0011673
35 Br	1.7407	0.9549	0.1574	987	0.0002412
38 Sr	3.4964	1.7492	0.2037	1914	0.0005960

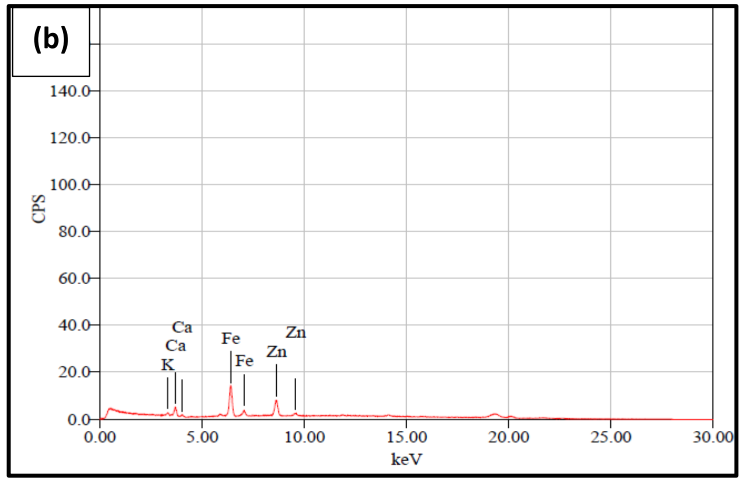
(b)

Quantitative result

Element	ms%	mol%	Sigma	Intensity
19 K	9.0068	9.9444	0.8574	902
20 Ca	64.8247	69.8262	0.6916	7927
26 Fe	26.1685	20.2294	0.2762	7005

Figure 4.12: Elemental analysis of Typha latifolia(shoots) in (a) August and (b)December





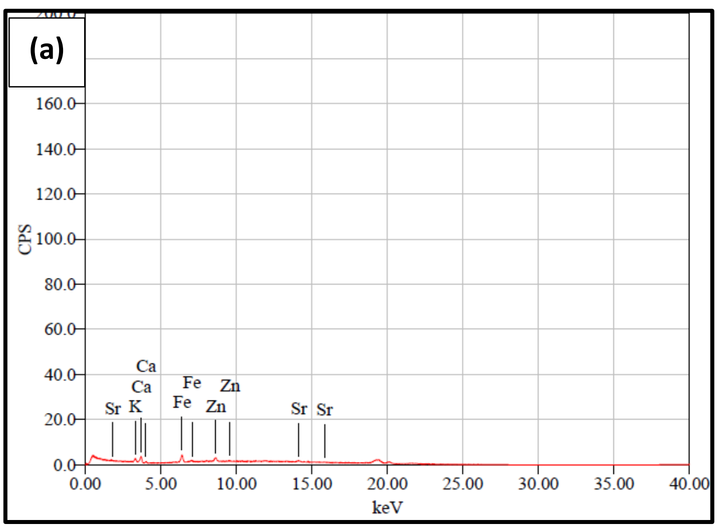
Quantitative result

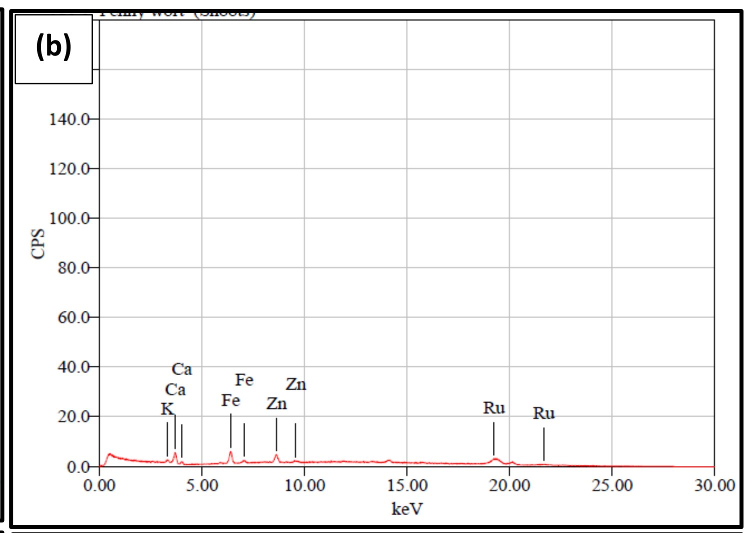
Element	ms%	mol%	Sigma	Intensity	K ratio	Line
19 K	5.9281	7.7653	0.4106	1111	0.0011941	K
20 Ca	27.9062	35.6629	0.2488	8500	0.0054573	K
22 Ti	1.7106	1.8292	0.1276	739	0.0002839	K
25 Mn	2.3280	2.1705	0.0771	1821	0.0004720	K
26 Fe	32.3477	29.6680	0.0723	29643	0.0066861	K
30 Zn	27.6202	21.6418	0.0955	22396	0.0047281	K
38 Sr	2.1592	1.2622	0.1558	1733	0.0005598	K

Quantitative result

Element	ms%	mol%	Sigma	Intensity	K ratio	Line
19 K	10.6570	13.5820	0.6543	1064	0.0010958	K
20 Ca	27.8760	34.6601	0.4365	4106	0.0025273	K
26 Fe	37.6871	33.6295	0.1232	17196	0.0037185	K
30 Zn	23.7798	18.1283	0.1633	9563	0.0019356	K

Figure 4.13: Elemental analysis of Centella asiatica (roots) in (a) August and (b)December





Quantitative result

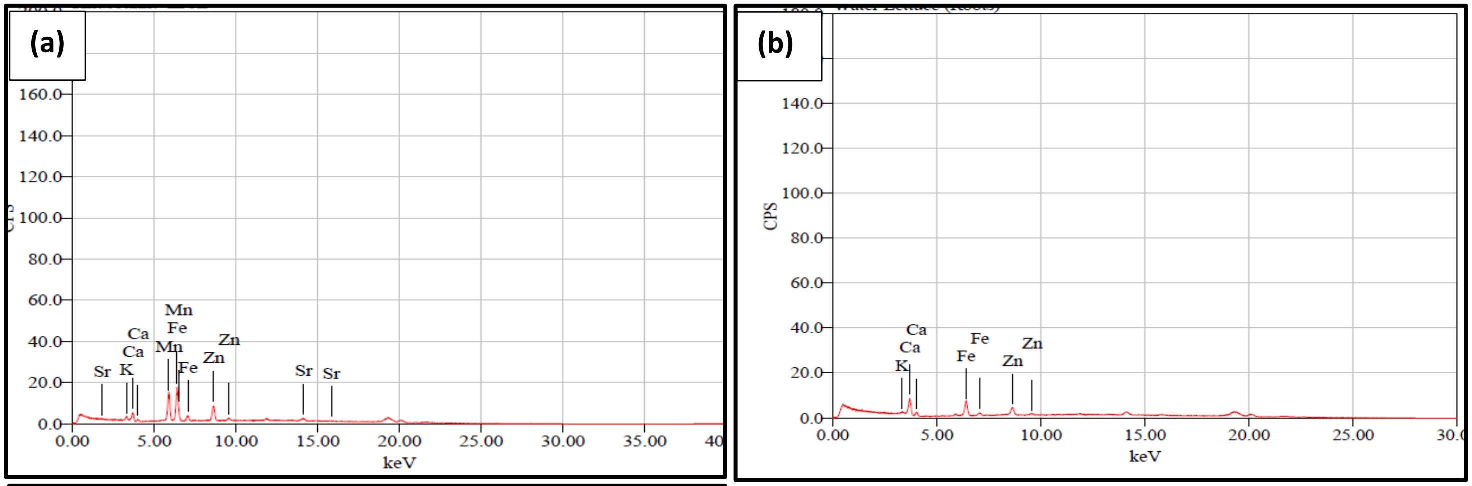
Element	ms%	mol%	Sigma	Intensity	K ratio	Line
19 K	25.2939	28.5792	0.8965	1536	0.0015578	K
20 Ca	44.9254	49.5217	0.8261	2914	0.0017659	K
26 Fe	18.8073	14.8785	0.2214	3980	0.0008473	K
30 Zn	8.6674	5.8579	0.2059	2305	0.0004592	K
38 Sr	2.3060	1.1628	0.2723	749	0.0002284	K

Quantitative result

Element	ms%	mol%	Sigma	Intensity	K ratio
19 K	15.8530	18.7465	0.9315	1198	0.0012411
20 Ca	46.5047	53.6508	0.7028	4584	0.0028376
26 Fe	20.0150	16.5716	0.1974	6144	0.0013360
30 Zn	11.8740	8.3990	0.1906	4410	0.0008976
44 Ru	5.7533	2.6321	1.0887	922	0.0012331

Figure 4.14: Elemental analysis of Centella asiatica(shoots) in (a) August and (b)December

(a)
(b)



Quantitative result

Element	ms%	mol%	Sigma	Intensity	K ratio	Line
19 K	11.2962	14.7972	0.4480	1793	0.0018854	K
20 Ca	18.0504	23.0676	0.2927	4316	0.0027119	K
25 Mn	26.4435	24.6541	0.0871	16916	0.0042904	K
26 Fe	25.2337	23.1433	0.0799	19331	0.0042668	K
30 Zn	16.3080	12.7781	0.1165	10007	0.0020674	K
38 Sr	2.6682	1.5598	0.1673	1842	0.0005823	K

Quantitative result

Element	ms%	mol%	Sigma	Intensity	K ratio
19 K	6.5359	7.6027	0.7747	649	0.0006711
20 Ca	55.5238	63.0108	0.5517	7617	0.0047085
26 Fe	25.1810	20.5086	0.2018	8259	0.0017938
30 Zn	12.7593	8.8779	0.2077	4749	0.0009654

Figure 4.15: Elemental analysis of Pistia stratiotes(shoots) in (a) August and (b)December

4.2 Comparative characterization of microbes of surface and sediments of wetlands and sludge of MBR system

Isolated strains KB 5, KB 6, KB 12, KB 13, KB 14 and KB 17 belong to the surface and sediments of Pond 2 while strains KB 1, KB 4, KB 8, KB 10, KB 11, KB 15, KB 16 and KB 19 belong to Pond 7 planted with *Centella asiatica*. Strains isolated and identified through activated sludge of pilot scale MBR system were KB 2, KB 3, KB 7, KB 9 and KB 18. The detail of identification of bacterial species is mentioned below:

4.2.1 Morphological characterization of isolates

4.2.1.1 Colony morphology

Table 4.1 represents the colony morphology of isolated strains (KB1-KB19). Colony morphology was studied in terms of form, color, elevation, margin, surface texture and opacity. Maximum percentage of strains had circular shape, white color, raised elevation, smooth texture and were opaque. Colony morphology is used to illustrate bacterial properties (Yildiz & Visick, 2009). Bacteria that form smooth colonies were capable of making more biofilms polysaccharides (Enos-Berlage & McCarter, 2000).

Table 4.1 : Colony morphology of bacterial strains isolated from surface and sediments of Phytoremediation system and activated sludge of MBR system

Strain ID	Source	Form	Color	Elevation	Margin	Surface texture	Opacity
KB 1	Wetlands	Circular	Offwhite	Flat	Filamentous	Smooth, moist	Opaque
KB 2	MBR Sludge	Punctiform	Offwhite	Raised	Erose	Dry	Opaque
KB 3	MBR Sludge	Irregular	Offwhite	Convex	Lobate	Dry, powdery	Opaque
KB 4	Wetlands	Round	Yellow	Flat	Entire	Smooth,moist	Opaque
KB 5	Wetlands	Irregular	Offwhite	Raised	Lobate	Smooth,moist	Opaque
KB 6	Wetlands	Circular	Offwhite	Raised	Entire	Smooth, moist	Opaque
KB 7	MBR Sludge	Negative	Single,chained	Bacillus	Positive	Negative	Opaque
KB 8	Wetlands	Round	Offwhite	Pulvinate	Entire, even	Smooth,moist	Opaque
KB 9	MBR Sludge	Round	Offwhite	Flat	Curled	Smooth,slimy	Opaque
KB10	Wetlands	Punctiform	Offwhite	Convex	Undulate	Smooth,moist	Opaque
KB 11	Wetlands	Filamentous	Offwhite	Raised	Filamentous	Smooth,moist	Opaque
KB 12	Wetlands	Irregular	Offwhite	Flat	Erose	Smooth,moist	Opaque

KB 13	Wetlands	Circular	Offwhite	Raised	Undulate	Smooth, moist	Opaque
KB 14	Wetlands	Circular	Yellow	Convex	Entire	Smooth, moist	Opaque
KB 15	Wetlands	Punctiform	Offwhite	Raised	Undulate	Smooth,glistening,moist	Opaque
KB 16	Wetlands	Round	Offwhite	Pulvinate	Curled	Smooth,moist	Opaque
KB 17	Wetlands	Irregular	Offwhite	Flat	Lobate	Dry, powdery	Opaque
KB 18	MBR Sludge	Round	Offwhite	Pulvinate	Undulate	Smooth,slimy	Opaque
KB 19	Wetlands	Irregular	Offwhite	Flat	Curled	Smooth,moist	Opaque

4.2.1.2 Cell morphology

Cell morphology of isolated bacterial strains in terms of gram reaction, shape, arrangement and motility is mentioned in detail in Table 4.2. All of the isolated strains were identified as gram negative and maximum percentage of bacteria had bacillus shape. Cell motility of maximum strains were observed when examined under 100X resolution of light microscope.

Table 4.2: Cell morphology of bacterial strains isolated from surface and sediments of Phytoremediation system and activated sludge of MBR system

Strain ID	Source	Gram reaction	Shape	Arrangement	Motility
KB 1	Wetlands	Negative	Bacillus	Single	Fast
KB 2	MBR Sludge	Negative	Bacillus	Paired	Fast
KB 3	MBR Sludge	Negative	Bacillus	Chain	Fast
KB 4	Wetlands	Negative	Straight rods	Single	Slow
KB 5	Wetlands	Negative	Bacillus	Single,paired	Fast
KB 6	Wetlands	Negative	Coci	Paired,chain	Fast
KB 7	MBR Sludge	Negative	Bacillus	Single	Non-motile
KB 8	Wetlands	Negative	Coci	Single,paired	Fast
KB 9	MBR Sludge	Negative	Bacillus	Single,paired	Fast
KB 10	Wetlands	Negative	Straight rods	Single	Slow

KB 11	Wetlands	Negative	Bacillus	Single,paired	Fast
KB 12	Wetlands	Negative	Bacillus(long rods)	Chain	Slow
KB 13	Wetlands	Negative	Bacillus	Single, paired	Slow
KB 14	Wetlands	Negative	Straight rods	Single	Fast
KB 15	Wetlands	Negative	Bacillus	Single,paired	Fast
KB 16	Wetlands	Negative	Bacillus	Single	Non-motile
KB 17	Wetlands	Negative	Bacillus	Single	Non-motile
KB 18	MBR Sludge	Negative	Bacillus	Paired	Slow
KB 19	Wetlands	Irregular	Off white	Flat	Curled

4.2.2 Biochemical characterization of isolates

After morphological characteriation, strains were subjected to biochemical characterization. The detail of which are mentioned in detail in Table 4.3. All of the strains to be identified showed the positive McConkey agar test and major percentage of strains had showed the positive oxidase, catalase and EMB agar test.

Table 4.3: Biochemical characterization of bacterial strains isolated from surface and sediments of Phytoremediation system and activated sludge of MBR system

Strain ID	Source	Oxidase	Catalase	EMB agar	McConkey agar
KB 1	Wetlands	Positive	Positive	Positive	Positive
KB 2	MBR Sludge	Negative	Positive	Positive	Positive
KB 3	MBR Sludge	Negative	Positive	Positive	Positive
KB 4	Wetlands	Negative	Negative	Positive	Positive
KB 5	Wetlands	Negative	Negative	Positive	Positive

KB 6	Wetlands	Positive	Negative	Positive	Positive
KB 7	MBR Sludge	Positive	Negative	Positive	Positive
KB 8	Wetlands	Negative	Negative	Positive	Positive
KB 9	MBR Sludge	Negative	Positive	Positive	Positive
KB 10	Wetlands	Negative	Positive	Negative	Positive
KB 11	Wetlands	Positive	Positive	Positive	Positive
KB 12	Wetlands	Positive	Negative	Positive	Positive
KB 13	Wetlands	Positive	Positive	Negative	Positive
KB 14	Wetlands	Negative	Positive	Negative	Positive
KB 15	Wetlands	Positive	Positive	Positive	Positive
KB 16	Wetlands	Positive	Positive	Positive	Positive
KB 17	Wetlands	Positive	Negative	Positive	Positive
KB 18	MBR Sludge	Negative	Positive	Negative	Positive
KB 19	Wetlands	Positive	Positive	Positive	Positive

4.2.3 Molecular characterization

Strains were characterized moleculary at genus and specie level through PCR amplification and 16S rRNA sequencig process. The detail of which are mentioned below:

4.2.3.1 DNA extraction and PCR amplification

DNA of the isolated strains were extracted through kit method and were confirmed by running it on agarose gel. Extracted DNA of the isolated strains were further subjected to PCR amplification process for the genus identification.

518 Forward and 800 Reverse primers were used to amplify the 500 bp fragment of 16S rRNA genes of isolated bacterial strains. PCR amplification products of 500 bp were obtained for all the isolates. Amplified genes of strains KB1-KB19 were visulaized by 1% agarose gel, stained with loading dye and was observed under UV transilluminator. Figure 4.16 is the gel picture of amplified genes of isolated strains.

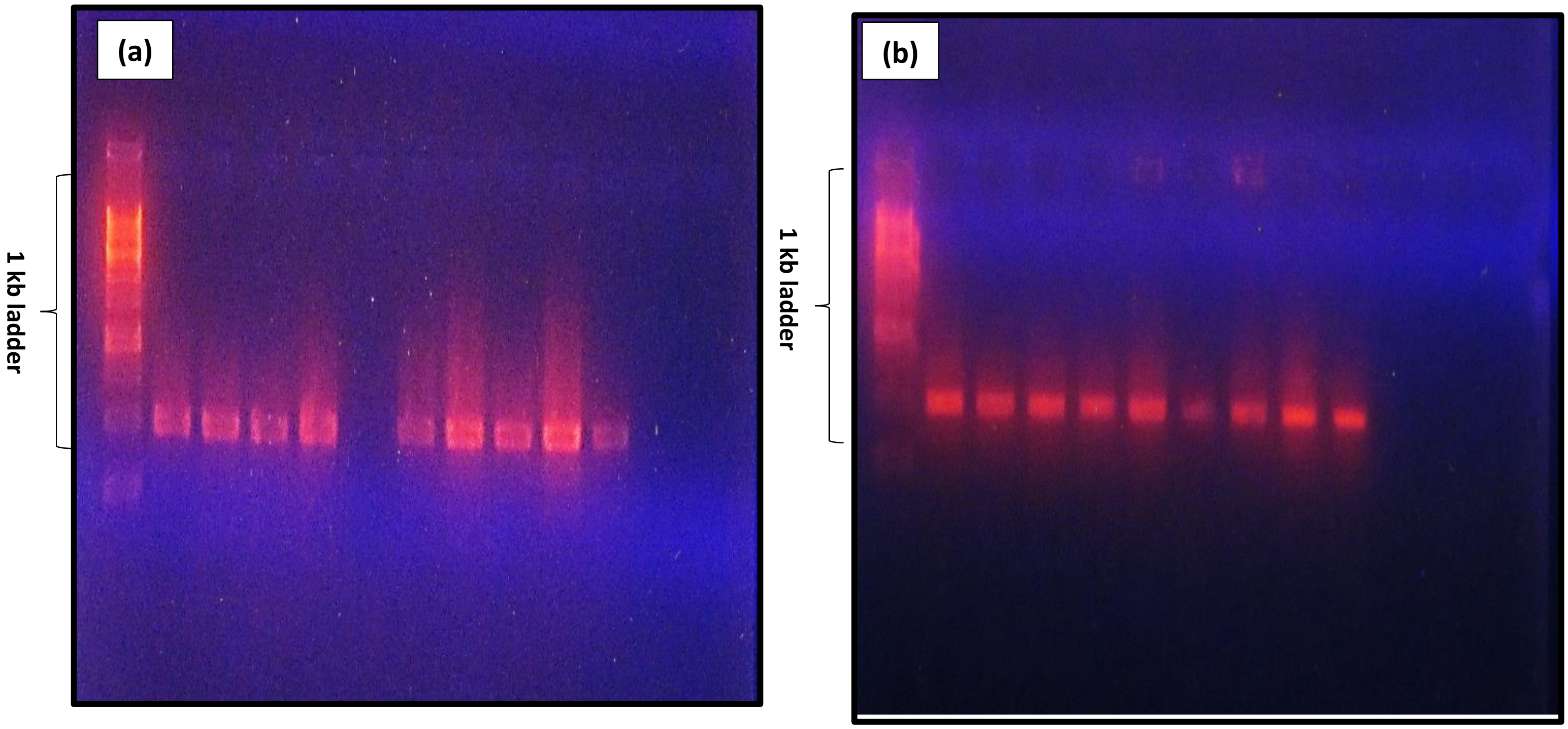


Figure 4.16: (a) From left to right, amplified genes of strains KB1-KB9 (b) from left to right, amplified genes of strains KB10-KB19

4.2.3.2 16S rRNA sequencing

PCR products were sent to genome analysis department, Macrogen. Sequences that were obtained were trimmed through Bio edit software and were identified through BLAST tool of NCBI. After getting the accession number phylogenetic tree (Figure 4.17) was constructed which demonstrate the relatedness and linkages of different bacterial strains identified.

Table 4.4: Source and scientific name of Identified species along with the accession number

Strain ID	Source	Specie Identified	Accession Number
K.B 1	Wetlands	*Enterobacter cloacae*	KY751345
K.B 2	MBR Sludge	*Pantoea dispersa*	KY751346
K.B 3	MBR Sludge	*Salmonella enterica*	KY751347
K.B 4	Wetlands	*Enterobacter kobei*	KY751348
K.B 5	Wetlands	*Escherichia coli*	KY751349
K.B 6	Wetlands	*Escherichia sp*	KY751350
K.B 7	MBR Sludge	*Shigella dysenteriae*	KY751351
K.B 8	Wetlands	*Escherichia coli*	KY751352
K.B 9	MBR Sludge	*Enterobacter hormaechei*	KY751353
K.B 10	Wetlands	*Franconibacter pulveris*	KY751354
K.B 11	Wetlands	*gamma proteobacterium*	KY751355
K.B 12	Wetlands	*Citrobacter freundii*	KY751356
K.B 13	Wetlands	*Enterobacter asburiae*	KY751357
K.B 14	Wetlands	*Enterobacter aerogenes*	KY751358
K.B 15	Wetlands	*Escherichia sp.*	KY751359
K.B 16	Wetlands	*Shigella sp*	KY751360
K.B 17	Wetlands	*Shigella dysenteriae*	KY751361
K.B 18	MBR Sludge	*Salmonella waycross*	KY751362
K.B 19	Wetlands	*Escherichia albertii*	KY751363

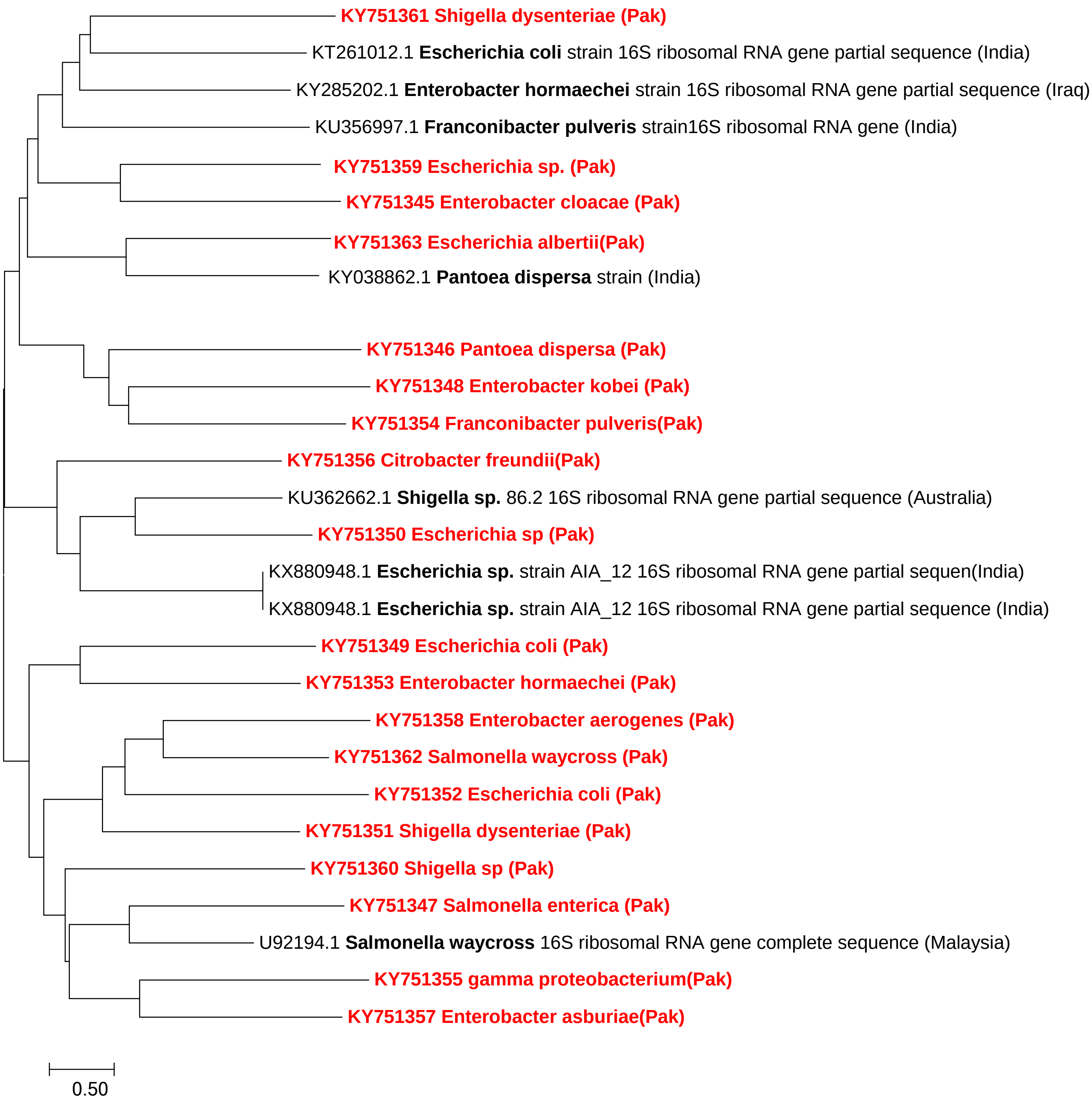


Figure 4.17: Phylogenetic tree demonstrating relatedness and linkage to different bacterial strains

The assessment of bacterial communities in CWs has been addressed by several researchers (Ibekwe *et al.,* 2003; Baptista *et al.,* 2003; Nicomrat *et al.,* 2006) and have categorized the microbial communities in CW for domestic wastewater and described that these systems are reliant on microbial compositions for optimum wastewater treatment. Dominant bacterial species isolated from Phytoremediation system belong to the Phylum proteobacteria the species identified were *Enterobacter cloacae, Enterobacter kobei, Enterobacter hormaechei, Enterobacter asburiae, Enterobacter aerogenes. gamma proteobacterium, Franconibacter pulveris, Citrobacter freundii, Shigella dysenteriae, Escherichia albertii* and *Escherichia coli.* This is perfectly in line with the study conducted by Calheiros and his coworkers in 2009 have worked on the identification of bacterial communities from wetlands and the results revealed γ-Proteobacteria being the most dominant phyla responsible for removal of phenols and organic compounds from wastewater. Previous studies have reported that aerobic autotrophic ammonia oxidizing bacteria, denitrifying bacteria and methanogens belong to the phyla proteobacteria and have an impressive role in pollutant removal from wetlands (Gorra *et al.,* 2007; Ibekwe *et al.,* 2007; Tietz *et al.,* 2007). Calheiros and his colleagues in 2009 have worked on the bacterial community dynamics of HSFCW and have identified *Firmicutes*, *Actinobacteria*, *α*, *β,* and *γ Proteobacteria* being the most dominant ones.

All bacterial species isolated from activated sludge of MBR system belong to the phyla proteobacteria. This is in accordance with the study conducted by Sato and his co-workers in 2016 and have identified α, β, and γ-proteobacteria as the most dominant species in pilot scale MBR system during operation with relevant abundance of 37% and have identified some species as organic substances consumers. Activated sludge of MBR system provides higher nutritious carbon and other features necessary for the development of a wide variety of microorganisms therefore, it has been recognized as an ideal source for isolation of targeted bacteria that are capable of enzyme degradation or production potentials (Karray *et al.,* 2016). Some previous studies have reported proteobacteria to be present in bulk in activated sludge and were associated with the secretion of EPS that is mainly responsible for formation of bio cake layer onto the membrane surface (Hu *et al*., 2012; Zhang *et al.,* 2012; Hu *et al.,* 2015).

4.3 Total biomass estimation of predominant macrophytes species

Root and shoot samples of predominant macrophytes were collected in August, September, December and January. Plants were analyzed in terms of total fresh and dry biomass production and relative growth rate. The detail of which are mentioned in detail below:

4.3.1 Total biomass

Fresh and dry weights of *Typha latifolia*, *Pistia stratiotes* and *Centella asiatica* revealed the highest fresh biomass production of 96250 kg per pond by *Typha latifolia* in the month of August followed by *Pistia stratiotes* and *Centella asiatica* i.e. 22599.5 and 1730.25 kg respectively. While the lowest fresh biomass production was noted in the month of January. In terms of dry biomass production same trend was observed as for fresh biomass. Figure 4.18 and 4.19 shows the trend of biomass production per pond by individual macrophytes under study. In previous studies, highest biomass production by *Typha latifolia* in spring and lowest by *Centella asiatica* were reported and biomass yield was stated in the range of 0.48 to 0.61 kg/m^2 and these values were found to be slightly higher than the present study due to the more optimum conditions provided. Relative growth rate was decreased with decrease in temperature for wetland plants (Atkinson *et al.,* 2010).

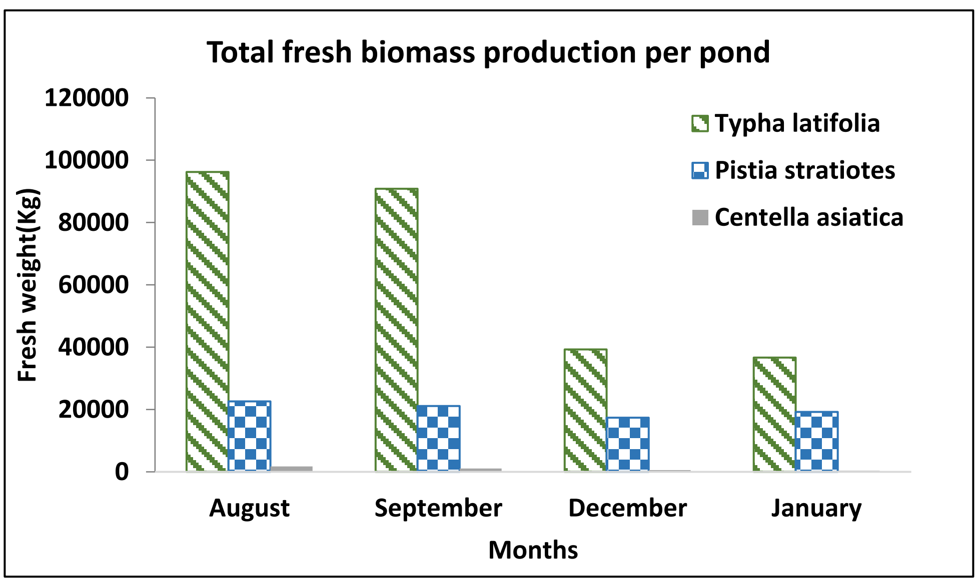


Figure 4.18: Monthly variations in fresh plant biomass production per pond

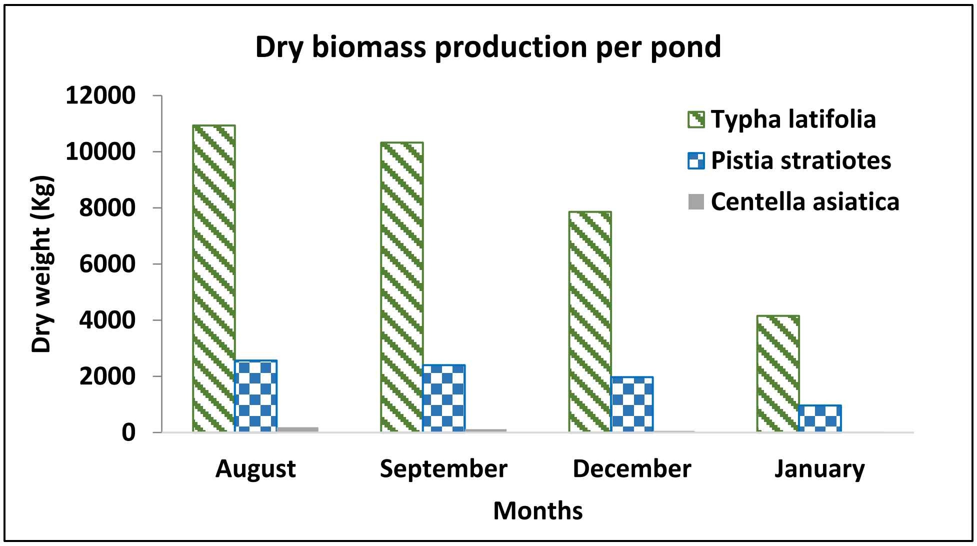


Figure 4.19: Monthly variations in dry plant biomass production per pond

4.3.2 Relative growth rate (%)

Relative decrease in the growth rate of plants were measured and *Typha latifolia* showed 93.5% decrease in the relative growth rate. Highest decrease in the relative growth rate of *Pistia stratiotes* were observed i.e. 97.9%. *Centella asiatica* had the lowest decrease in the relative growth rate and it was 74.5%. *Typha latifolia* had the decrease in relative growth of 93.5%. This is demonstrated in Figure 4.20.

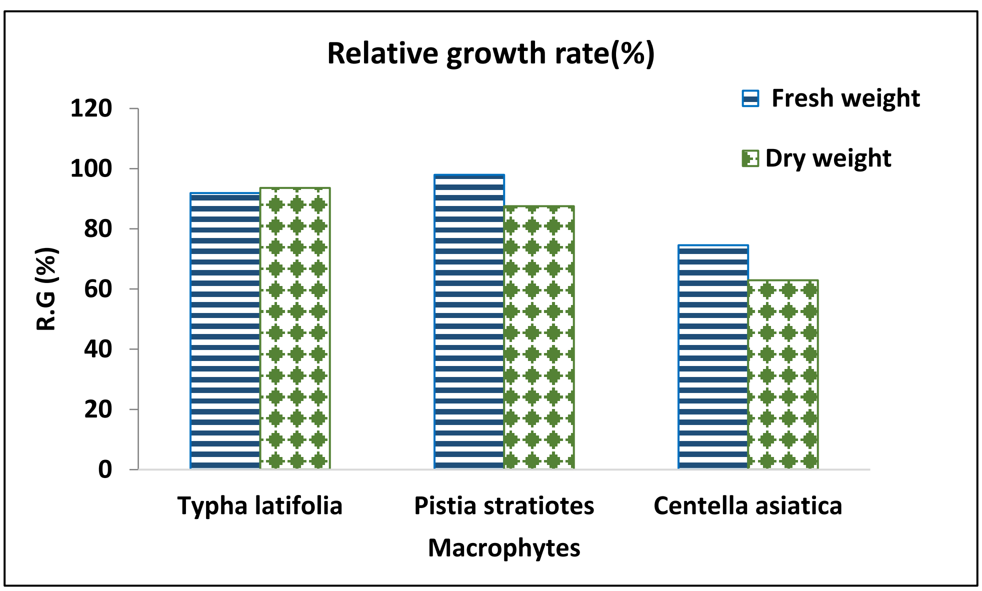


Figure 4.20: Relative growth rate of plants

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

CWs and MBR systems are efficient and reliable techniques for removal of pollutants from domestic wastewater and reusing it for irrigation purposes.

The main conclusions are:

1. Higher efficiency of MBR system than phytoremediation system was observed. Climatic parameters have strong positive and negative correlation with water quality parameters relevant to Constructed Wetlands. Ambient temperature and Global Horizontal irradiance were negatively correlated with water quality parameters ($r > -0.7$). While, Wind speed and Air pressure were positively correlated with water quality parameters ($r > 0.7$). Concerning Relative Humidity, non-significant correlation was noted ($r = 0$).
2. Removal efficiency of phytoremediation system was 91.5, 90.8, 67.7, 66.9 and 90 % for Turbidity, TSS, COD, BOD and Coliform removal respectively. While removal efficiency of MBR system was 99, 99, 83, 100 and 99 % for Turbidity, TSS, COD, BOD and Coliform removal respectively.
3. Elemental uptake was highest in the month of August whereas lowest in December. Roots accumulated more elements than shoots. Highest elements were uptaken by roots of *Typha latifolia* (Typha) followed by roots of *Centella asiatica (Penny wort)* and roots of *Pistia stratiotes* (Water lettuce).
4. Predominant species identified from wastewater of Phytoremediation system belongs to the phyla Proteobacterium (*Enterobacter cloacae, Enterobacter kobei*, *Enterobacter hormaechei*, *Enterobacter asburiae*, *Enterobacter aerogenes. gamma proteobacterium, Franconibacter pulveris, Citrobacter freundii*, *Shigella dysenteriae*, *Escherichia albertii and Escherichia coli*). While, predominant bacterial species identified from activated sludge of MBR system were *Salmonella enterica*, *Pantoea dispersa, Shigella dysenteriae*, *Enterobacter hormaechei* and *Salmonella waycross*, and they too belong to the phyla proteobacteria.

5. Net biomass production was highest by *Typha latifolia* (96250 kgs/pond) followed by Centella asiatica (1730.25 kg/pond) in growth. Highest decrease in the relative growth rate of *Pistia stratiotes* (Water lettuce) were noted i.e. 97.9% followed by 93.5% decrease in relative growth rate of *Typha latifolia*.

In conclusion, Phytoremediation came out to be a suitable technology for treating domestic wastewater, land limitation was a major issue that needs to be considered before policy making however, lesser energy requirements and low capital cost were the bigger advantage for the decision makers to take into consideration. MBR system came out to be reliable in terms of better effluent water quality, lesser sludge production and lower land requirements however operational cost and maintenance requirements remained a bottleneck in the overall performance that need to be figured out.

5.2 Recommendations

1. Enzymatic and microbial degradation pathway studies of bacterial community may be examined in detail for better performance efficiency of the systems.
2. Comparison of both MBR and Phytoremediation system with Conventional wastewater treatment technologies may be carried out.
3. Relationship between methane emission and organic matter removal from constructed wetland may be analyzed.
4. Elemental analysis of soil sediments of constructed wetlands may be carried out.
5. Inoculation of beneficial bacteria (already identified) in both Phytoremediation and MBR systems, to check improvements in the performance efficiencies at both lab and pilot scales.
6. Effects of different hydraulic rates on pollutant removal efficiency of phytoremediation system may be carried out.
7. Analysis of heavy metal uptake by macrophytes of Phytoremediation system may be carried out by using Atomic absorption spectrophotometer.
8. Hybridization of Phytoremediation and MBR system with microbial fuel cells at lab scale to check the enhanced pollutant removal efficiency of both systems.
9. Identification of isolated bacterial species in advanced PCR-DGGE.

6. REFERENCES

Abe, H., Katayama, A., Sah, B. P., Toriu, T., Samy, S., Pheach, P.,& Grierson, P. F. (2007). Potential for rural electrification based on biomass gasification in Cambodia. *Biomass and Bioenergy,* 31(9), 656-664.

Adrados, B. Á. R. B. A. R. A., Sánchez, O. L. G. A., Arias, C. A., Becares, E. L. O. Y., Garrido, L. A. U. R. A., Mas, J. O. R. D. I., ... & Morató, J. O. R. D. I. (2014). Microbial communities from different types of natural wastewater treatment systems: vertical and horizontal flow constructed wetlands and biofilters. *Water Research,* 55(4), 304-312.

Ahmed, Z., Cho, J., Lim, B. R., Song, K. G., & Ahn, K. H. (2007). Effects of sludge retention time on membrane fouling and microbial community structure in a membrane bioreactor. *Journal of Membrane Science*, 287(2), 211-218.

Ahsan, S., Rahman, M. A., Kaneco, S., Katsumata, H., Suzuki, T., & Ohta, K. (2005). Effect of temperature on wastewater treatment with natural and waste materials. *Clean Technologies and Environmental Policy,* 7(3), 198-202.

Akratos, C. S., & Tsihrintzis, V. A. (2007). Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. *Ecological Engineering,* 29(2), 173-191.

Akratos, C. S., Papaspyros, J. N., & Tsihrintzis, V. A. (2009). Total nitrogen and ammonia removal prediction in horizontal subsurface flow constructed wetlands: use of artificial neural networks and development of a design equation. *Bioresource Technology*, 100(2), 586-596.

Alfred, B. E. (2011). Gram staining technique. Longman Private Limited.

Ali, M. H. (2008). Assessment of some water quality characteristics and determination of some heavy metals in Lake Manzala, Egypt. *J. Aquat. Biol & Fish*, *5*(3), 17-30.

APHA, A. W. (2017). Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association & Water Environment Federation. (EW Rice, RB Baird, AD Eaton, & LS Clesceri, Ed.)(22nd ed.). Washington.

Atkinson, R. B., Perry, J. E., Noe, G. B., Daniels, W. L., & Cairns, J. (2010). Primary productivity in 20-year old created wetlands in southwestern Virginia. *Wetlands*, 30(2), 200-210.

Baig, S. A., Huang, L., Sheng, T., Lv, X., Yang, Z., Qasim, M., & Xu, X. (2017). Impact of climate factors on cyanobacterial dynamics and their interactions with water quality in South Taihu Lake, China. *Chemistry and Ecology*, 33(1), 76-87.

Baptista, J. (2003, May). Microbial communities in subsurface flow wetlands. In 1st International Seminar on the use of aquatic macrophytes for wastewater treatment in constructed wetlands (Vol. 810).

Buer, T., & J. Cumin (2010), MBR module design and operation, *Desalination*, 250(3), 1073-1077.

Calheiros, C. S. C., Teixeira, A., Pires, C., Franco, A. R., Duque, A. F., Crispim, L. F. C., ... & Castro, P. M. L. (2010). Bacterial community dynamics in horizontal flow constructed wetlands with different plants for high salinity industrial wastewater polishing. *Water Research*, 44(17), 5032-5038.

Calheiros, C. S., Duque, A. F., Moura, A., Henriques, I. S., Correia, A., Rangel, A. O., & Castro, P. M. (2009). Substrate effect on bacterial communities from constructed wetlands planted with Typha latifolia treating industrial wastewater. *Ecological Engineering*, 35(5), 744-753.

Chae, S. R., & Shin, H. S. (2007). Characteristics of simultaneous organic and nutrient removal in a pilot-scale vertical submerged membrane bioreactor (VSMBR) treating municipal wastewater at various temperatures. *Process Biochemistry* 42(2):193-198.

Chae, S.R.; Ahn, Y.; Hwang, Y.; Jang, D.; Meng, F.; Shi, J.; Lee, S.H.; Shin, H.S. Cheesbrough, M. (2006). District laboratory practice in tropical countries. *Cambridge University Press.*

|

Chen, Y., Wen, Y., Zhou, Q., & Vymazal, J. (2014). Effects of plant biomass on denitrifying genes in subsurface-flow constructed wetlands. *Bioresource Technology*, 157, 341-345.

Choi, J., Kim, E. S., & Ahn, Y. (2017). Microbial community analysis of bulk sludge/cake layers and biofouling-causing microbial consortia in a full-scale aerobic membrane bioreactor. *Bioresource Technology*, 227, 133-141.

Choukr-Allah, R., & Hamdy, A. (2005). Wastewater treatment and reuse as a potential water resource for irrigation. The use of non-conventional water resources. Bari: CIHEAM/EU DG Research, 101-124.

Ciria, M. P., Solano, M. L., & Soriano, P. (2005). Role of macrophyte Typha latifolia in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel. *Biosystems Engineering*, 92(4), 535-544.

Cooper, P. F., & Findlater, B. C. (Eds.). (2013). Constructed Wetlands in Water Pollution Control: Proceedings of the International Conference on the Use of Constructed Wetlands in Water Pollution Control, Held in Cambridge, UK, 24–28 September 1990. *Elsevier*.

Csavina, J., Field, J., Félix, O., Corral-Avitia, A. Y., Sáez, A. E., & Betterton, E. A. (2014). Effect of wind speed and relative humidity on atmospheric dust concentrations in semi-arid climates. *Science of the Total Environment*, 487, 82-90.

Dan, T. H., Chiem, N. H., & Brix, H. (2011). Treatment of high-strength wastewater in tropical constructed wetlands planted with Sesbania sesban: horizontal subsurface flow versus vertical downflow. *Ecological Engineering*, 37(5), 711-720.

de Jonge, V. N., Schuttelaars, H. M., van Beusekom, J. E., Talke, S. A., & de Swart, H. E. (2014). The influence of channel deepening on estuarine turbidity levels and dynamics, as exemplified by the Ems estuary. Estuarine, Coastal and Shelf Science, 139, 46-59.

Enos-Berlage, J. L., & McCarter, L. L. (2000). Relation of capsular polysaccharide production and colonial cell organization to colony morphology in Vibrio parahaemolyticus. *Journal of Bacteriology*, 182(19), 5513-5520.

Faulwetter, J.L., Gagnon, V. C., Sundberg, F., Chazarenc, M.D., Brisson, J. Camper, A.K. & Stein, O.R. (2009). Microbial processes influencing performance of treatment wetlands: a review. *Ecological Engineering* 35, 987–1004

Fawole, M. O., & Oso, B. A. (2004). Characterization of bacteria. *Laboratory Manual of Microbiology*. Spectrum Books Limited, Nigeria, 22-33.

Feng, P., Weagant, S. D., & Jinneman, K. (2014). BAM: diarrheagenic Escherichia coli.

Fishar, M. R. A. (1999). Distribution and abundance of benthic assemblages in El-Gamil basin(Lake Manzalah, Egypt). B. macrobenthos. *Bulletin of the National Institute of Oceanography and Fisheries(Egypt)*, *25*, 167-180.

Fountoulakis, M. S., Sabathianakis, G., Kritsotakis, I., Kabourakis, E. M., & Manios, T. (2017). Halophytes as vertical-flow constructed wetland vegetation for domestic wastewater treatment. *Science of the Total Environment*, *583*, 432-439.

Gorra, R., Coci, M., Ambrosoli, R., & Laanbroek, H. J. (2007). Effects of substratum on the diversity and stability of ammonia-oxidizing communities in a constructed wetland used for wastewater treatment. *Journal of Applied Microbiology*, 103(5), 1442-1452.

Gurung, K., Ncibi, M. C., & Sillanpää, M. (2017). Assessing membrane fouling and the performance of pilot-scale membrane bioreactor (MBR) to treat real municipal wastewater during winter season in Nordic regions. *Science of the Total Environment*, 579, 1289-1297.

Hai, F. I., Riley, T., Shawkat, S., Magram, S. F., and Yamamoto, K. (2014). Removal of pathogens by membrane bioreactors: a review of the mechanisms, influencing factors and reduction in chemical disinfectant dosing. *Water*, 6(12):3603-3630.

Hashisho, J., and El-Fadel, M. (2016). Membrane bioreactor technology for leachate treatment at solid waste landfills. *Reviews in Environmental Science and Bio/Technology* 15(3):441-463.

Hasnain, G., 2016. Membrane bioreactor technology for wastewater reclamation and reuse (Doctoral dissertation). Retrieved from IESE library database.

Haydar, S., Haider, H., Nadeem, O., Hussain, G., & Zahra, S. (2015). Proposed model for wastewater treatment in Lahore using constructed wetlands. *Journal of Faculty of Engineering & Technology*, 22(1), 9-19.

He, M. X., Hu, Q., Zhu, Q., Pan, K., & Li, Q. (2015). The feasibility of using constructed wetlands plants to produce bioethanol. *Environmental Progress & Sustainable Energy*, 34(1), 276-281.

Hellawell, J. M. (Ed.). (2012). Biological indicators of freshwater pollution and environmental management. *Springer Science & Business Media*.

Herrera, A. (2015). Responses to flooding of plant water relations and leaf gas exchange in tropical tolerant trees of a black-water wetland. Plant Responses Flooding, 4(106), 6.

Hu, D., Zhou, Z., Shen, X., Wei, H., Jiang, L. M., & Lv, Y. (2015). Effects of alkalinity on membrane bioreactors for reject water treatment: Performance improvement, fouling mitigation and microbial structures. *Bioresource Technology*, 197, 217-226.

Hu, M., Wang, X., Wen, X., & Xia, Y. (2012). Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. *Bioresource Technology*, 117, 72-79.

Hülsen, T., Barry, E. M., Lu, Y., Puyol, D., Keller, J., & Batstone, D. J. (2016). Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. *Water Research*, 100, 486-495.

Ibekwe, A. M., Grieve, C. M., & Lyon, S. R. (2003). Characterization of microbial communities and composition in constructed dairy wetland wastewater effluent. *Applied and Environmental Microbiology*,69(9), 5060-5069.

Ibekwe, A. M., Lyon, S. R., Leddy, M., & Jacobson-Meyers, M. (2007). Impact of plant density and microbial composition on water quality from a free water surface constructed wetland. *Journal of Applied Microbiology*, 102(4), 921-936.

Iorhemen, O. T., Hamza, R. A., & Tay, J. H. (2016). Membrane bioreactor (MBR) technology for wastewater treatment and reclamation: membrane fouling. *Membranes*, *6*(2), 33.

Jianquan, L., L.uhui, D., (2011). Influence of pH on treatment of dairy wastewater by nanofiltration using shear-enhanced filtration system. *Desalination*, 150-156.

Jouanneau, S., Recoules, L., Durand, M. J., Boukabache, A., Picot, V., Primault, Y., ... & Thouand, G. (2014). Methods for assessing biochemical oxygen demand (BOD): a review. *Water Research*, 49, 62-82.

Judd, S. (2008). The status of membrane bioreactor technology. *Trends in Biotechnology*, 26(2), 109-116.

Kadlec, R. H., & Wallace, S. (2009). Treatment wetlands. CRC press.

Kann, J., & Welch, E. B. (2005). Wind control on water quality in shallow, hypereutrophic Upper Klamath Lake, Oregon. *Lake and Reservoir Management*, 21(2), 149-158.

Karray, F., Mezghani, M., Mhiri, N., Djelassi, B., & Sayadi, S. (2016). Scale-down studies of membrane bioreactor degrading anionic surfactants wastewater: Isolation of new anionic-surfactant degrading bacteria. *International Biodeterioration & Biodegradation*, 114, 14-23.

Lagomarsino L, Diovisalvi N, Bustingorry J, Escaray R, & Zagarese HE (2015) Diel patterns of total suspended solids, turbidity, and water transparency in a highly turbid, shallow lake (Laguna Chascomús, Argentina). *Hydrobiologia*,752(1):21–31

Latif, U., & Dickert, F. L. (2015). Chemical Oxygen Demand. In Environmental Analysis by Electrochemical Sensors and Biosensors (pp. 719-728). *Springer New York.*

Lave, M., Hayes, W., Pohl, A., & Hansen, C. W. (2015). Evaluation of global horizontal irradiance to plane-of-array irradiance models at locations across the United States. *IEEE journal of Photovoltaics*, 5(2), 597-606.

Lesjean, B., & Gnirss, R. (2006). Grey water treatment with a membrane bioreactor operated at low SRT and low HRT. *Desalination*, 199(1-3), 432-434.

Li, Y., Liu, K., Wang, Y., Zhou, Z., Chen, C., Ye, P., & Yu, F. (2018). Improvement of cadmium phytoremediation by Centella asiatica L. after soil inoculation with cadmium-resistant Enterobacter sp. FM-1. *Chemosphere*.

Liberman, N., Shandalov, S., Forgacs, C., Oron, G., & Brenner, A. (2016). Use of MBR to sustain active biomass for treatment of low organic load grey water. *Clean Technologies and Environmental Policy*, 18(4), 219-1224.

Lin, H., Zhang, M., Wang, F., Meng, F., Liao, B. Q., Hong, H., ... & Gao, W. (2014). A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: characteristics, roles in membrane fouling and control strategies. *Journal of Membrane Science* 460, 110-125.

Liu, R., Huang, X., Chen, L., Wen, X., & Qian, Y. (2005). Operational performance of a submerged membrane bioreactor for reclamation of bath wastewater. *Process Biochemistry*, 40(1), 125-130.

Luo, W., Phan, H. V., Hai, F. I., Price, W. E., Guo, W., Ngo, H. H., ... & Nghiem, L. D. (2016). Effects of salinity build-up on the performance and bacterial community structure of a membrane bioreactor. *Bioresource Technology*, 200, 305-310.

Ma, Z., Wen, X., Zhao, F., Xia, Y., Huang, X., Waite, D., & Guan, J. (2013). Effect of temperature variation on membrane fouling and microbial community structure in membrane bioreactor. *Bioresource Technology*, 133, 462-468.

Major, M., & Cieśliński, R. (2017). Impact of hydrometeorological conditions on the chemical composition of water in closed-basin kettle ponds: a comparative study of two postglacial areas. *Journal of Elementology*, *22*(1), 151-167.

Mancilla Vi, R., Zúñiga, J., Salgado, E., Schiappacasse, M. C., & Chamy Maggi, R. (2013). Constructed wetlands for domestic wastewater treatment in a Mediterranean climate region in Chile. *Electronic Journal of Biotechnology* 16(4):5-5.

Mander, Ü., Tournebize, J., Kasak, K., & Mitsch, W. J. (2014). Climate regulation by free water surface constructed wetlands for wastewater treatment and created riverine wetlands. *Ecological Engineering*, 72, 103-115.

Mara, D. (2013). Domestic wastewater treatment in developing countries. Routledge.

Marguí, E., Hidalgo, M., & Queralt, I. (2007). XRF spectrometry for trace element analysis of vegetation samples. *Spectroscopy Europe*, 19(3), 13-18.

Meng, P., Pei, H., Hu, W., Shao, Y., & Li, Z. (2014). How to increase microbial degradation in constructed wetlands: influencing factors and improvement measures. *Bioresource Technology*, 157, 316-326.

Merz, C., Scheumann, R., El Hamouri, B., & Kraume, M. (2007). Membrane bioreactor technology for the treatment of greywater from a sports and leisure club. *Desalination,* 215(1-3), 37-43.

Mustapha A, Aris AZ, Juahir H, Ramli MF, Kura NU (2013a) River water quality assessment using environmentric techniques: case study of Jakara River Basin. *Environmental Science and Pollution Research*, 20(8), 5630–5644

Mutamim, N. S. A., Noor, Z. Z., Hassan, M. A. A., Yuniarto, A., & Olsson, G. (2013). Membrane bioreactor: applications and limitations in treating high strength industrial wastewater. *Chemical Engineering Journal*, 225, 109-119.

Neoh, C. H., Noor, Z. Z., Mutamim, N. S. A., & Lim, C. K. (2016). Green technology in wastewater treatment technologies: integration of membrane bioreactor with various wastewater treatment systems. *Chemical Engineering Journal*, 283, 582-594.

Nicomrat, D., Dick, W. A., & Tuovinen, O. H. (2006). Assessment of the microbial community in a constructed wetland that receives acid coal mine drainage. *Microbial Ecology*, 51(1), 83-89.

Oh, H. S., Tan, C. H., Low, J. H., Rzechowicz, M., Siddiqui, M. F., Winters, H., ... & Rice, S. A. (2017). Quorum quenching bacteria can be used to inhibit the biofouling of reverse osmosis membranes. *Water Research*, 112, 29-37.

Olutiola, P. O., Famurewa, O., & Sontag, H. G. (2000). An Introduction to General Microbiology: A Practical Approach. HeidebergerVerlagsanstait and DruckereiGmb.

Oon, Y. L., Ong, S. A., Ho, L. N., Wong, Y. S., Oon, Y. S., Lehl, H. K., and Thung, W. E. (2015). Hybrid system up-flow constructed wetland integrated with microbial fuel cell for simultaneous wastewater treatment and electricity generation. *Bioresource Technology,* 186, 270-275.

Oopkaup, K., Truu, M., Nõlvak, H., Ligi, T., Preem, J. K., Mander, Ü., & Truu, J. (2016). Dynamics of Bacterial Community Abundance and Structure in Horizontal Subsurface Flow Wetland Mesocosms Treating Municipal Wastewater. *Water*, 8(10), 457.

Orebiyi, O. & Awomeso, A. (2008). Water and pollution agents in the 21st century. *Nature and Science,* 6, 16–24.

Oschmann, N., Nghiem, L. D., & Schäfer, A. I. (2005). Fouling mechanisms of submerged ultrafiltration membranes in greywater recycling. *Desalination*, 179(1-3), 215-223.

Pott, V. J., & Pott, A. (2002). *Potencial de uso de plantas aquáticas na despoluição da água*. Embrapa Gado de corte.

Qu, F., Liang, H., Zhou, J., Nan, J., Shao, S., Zhang, J., & Li, G. (2014). Ultrafiltration membrane fouling caused by extracellular organic matter (EOM) from Microcystis aeruginosa: effects of membrane pore size and surface hydrophobicity. *Journal of Membrane Science*. 449, 58-66.

Rai, U. N., Tripathi, R. D., Singh, N. K., Upadhyay, A. K., Dwivedi, S., Shukla, M. K., ... & Nautiyal, C. S. (2013). Constructed wetland as an ecotechnological tool for pollution treatment for conservation of Ganga river. *Bioresource Technology*, 148, 535-541.

Rashidi, H., GhaffarianHoseini, A., GhaffarianHoseini, A., Sulaiman, N. M. N., Tookey, J., & Hashim, N. A. (2015). Application of wastewater treatment in sustainable design of green built environments: A review. *Renewable and Sustainable Energy Reviews*, *49*, 845-856.

Robles-Pliego, M., Olguín, E. J., Hernández-Landa, J., González-Portela, R. E., Sánchez-Galván, G., & Cuervo-López, F. (2015). Dual Purpose System for Water Treatment From a Polluted River and the Production of Pistia stratiotes Biomass Within a Biorefinery. *CLEAN–Soil, Air, Water*, *43*(11), 1514-1521.

Saeed, T., & Sun, G. (2013). A lab-scale study of constructed wetlands with sugarcane bagasse and sand media for the treatment of textile wastewater. *Bioresource Technology*, 128, 438-447.

Salem, Z. B., Laffray, X., Al-Ashoor, A., Ayadi, H., & Aleya, L. (2017). Metals and metalloid bioconcentrations in the tissues of Typha latifolia grown in the four interconnected ponds of a domestic landfill site. *Journal of Environmental Sciences*, *54*, 56-68.

Sander, R. (2015). Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmospheric Chemistry & Physics*, *15*(8).

Sarma, B., & Goswami, B. C. (2016). Qualitative elemental analysis of some selected antidiabetic medicinal plants of Assam using X-ray Fluorescence (XRF) technique. *Asian Journal of Plant Science and Research*, 6(3), 71-79.

Sastroutomo, S. S., Ikusima, I., & Numata, M. (1978). Ecological studies of water hyacinth (Eichhornia crassipes Mart. Solms) with special emphasis on their growth. *Japanese Journal of Ecology*, 28(3), 191-197.

Sankararajan, V., Neelakandhan, N., & Chandrasekaran, S. (2017). Modeling of constructed wetland performance in BOD5 removal for domestic wastewater under changes in relative humidity using genetic programming. *Environmental Monitoring and Assessment*, *189*(4), 164.

Sato, Y., Hori, T., Navarro, R. R., Habe, H., Yanagishita, H., & Ogata, A. (2016). Fine-scale monitoring of shifts in microbial community composition after high organic loading in a pilot-scale membrane bioreactor. *Journal of Bioscience and Bioengineering*, 121(5), 550-556.

Sharma, D. & Dubey, A. (2011). Seasonal Study of Nutritional Status for Microcystis Aeruginosa and Water Hyacianth of Water bodies of Indore, India. *Annals of Biological Research*, 2, 461–468.

Shin, C., McCarty, P. L., Kim, J., & Bae, J. (2014). Pilot-scale temperate-climate treatment of domestic wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR). *Bioresource Technology*, 159, 95-103.

Sleytr, K., Tietz, A., Langergraber, G., Haberl, R., & Sessitsch, A. (2009). Diversity of abundant bacteria in subsurface vertical flow constructed wetlands. *Ecological Engineering*, 35(6), 1021-1025.

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., ... & Moormann, H. (2003). Effects of plants and microorganisms in constructed wetlands for wastewater treatment. *Biotechnology Advances*, 22(1), 93-117.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725-2729.

Tanaka, N., Jinadasa, K. B. S. N., Werellagama, D. R. I. B., Mowjood, M. I. M., & Ng, W. J. (2006). Constructed tropical wetlands with integrated submergent-emergent plants for sustainable water quality management. *Journal of Environmental Science and Health Part A*, 41(10), 2221-2236.

Tietz, A., Kirschner, A., Langergraber, G., Sleytr, K., & Haberl, R. (2007). Characterisation of microbial biocoenosis in vertical subsurface flow constructed wetlands. *Science of the Total Environment*, 380(1), 163-172.

Tortora, G. J., Funke, B. R., & Case, C. L. (2004). Functional anatomy of prokaryotic and eukaryotic cells. *Microbiology-An Introduction*. Pearson Education, 113-118.

Verma, A., Wei, X., & Kusiak, A. (2013). Predicting the total suspended solids in wastewater: a data-mining approach. *Engineering Applications of Artificial Intelligence*, 26(4), 1366-1372.

Victor, K. K., Séka, Y., Norbert, K. K., Sanogo, T. A., & Celestin, A. B. (2016). Phytoremediation of wastewater toxicity using water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes). *International Journal of Phytoremediation*, 18(10), 949-955.

Vigneswaran, S., & Sundaravadivel, M. (2014). Wastewater recycle, reuse, and reclamation–recycle and reuse of domestic wastewater. *Encyclopedia of Life Support Systems*, 1.

Vymazal, J. (2009). The use constructed wetlands with horizontal sub-surface flow for various types of wastewater. *Ecological Engineering, 35*(1), 1-17.

Vymazal, J. (2011). Plants used in constructed wetlands with horizontal subsurface flow: a review. *Hydrobiologia,* 674(1), 133-156.

Vymazal, J. (2013). Emergent plants used in free water surface constructed wetlands: a review. *Ecological Engineering*, 61, 582-592.

Vymazal, J., & Březinová, T. (2014). Long term treatment performance of constructed wetlands for wastewater treatment in mountain areas: four case studies from the Czech Republic. *Ecological Engineering*, 71, 578-583.

Waheed, H., Hashmi, I., Naveed, A. K., & Khan, S. J. (2013). Molecular detection of microbial community in a nitrifying–denitrifying activated sludge system. *International Biodeterioration & Biodegradation*, 85, 527-532.

Wakelin, S.A., Colloff, M.J., & Kookana, R.S. (2008). Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable season flow. *Applied and Environmental Microbiology*, 74, 2659–2668.

Wang, Q., Xie, H., Ngo, H. H., Guo, W., Zhang, J., Liu, C., ... & Zhao, C. (2016). Microbial abundance and community in subsurface flow constructed wetland microcosms: role of plant presence. *Environmental Science and Pollution Research*, 23(5), 4036-4045.

Wanninkhof, R. (2014). Relationship between wind speed and gas exchange over the ocean revisited. *Limnol. Oceanogr. Methods,* 12(6), 351-362.

Weerakoon, G. M. P. R., Jinadasa, K. B. S. N., Herath, G. B. B., Mowjood, M. I. M., Zhang, D., Tan, S. K., & Jern, N. W. (2016). Performance of tropical vertical subsurface flow constructed wetlands at different hydraulic loading rates." *CLEAN–Soil, Air, Water* 44(8), 938-948.

Wood, T. M., & Cheng, R. T. (2006, September). Use of UnTRIM to investigate dissolved oxygen transport in Upper Klamath Lake, Oregon. In Proceedings of the 7th international conference on hydroscience and engineering. College of Engineering, Drexel University, Philadelphia. Available: hdl. handle. net/1860/1424.(February 2009) (pp. 1-12).

World Bank, 2012. http://data.worldbank.org/ (accessed 28.02.14). World Health Organization, 2012. *Progress on Drinking Water and Sanitation.*

Wu, H., Zhang, J., Ngo, H. H., Guo, W., Hu, Z., Liang, S., ... & Liu, H. (2015). A review on the sustainability of constructed wetlands for wastewater treatment: design and operation. *Bioresource Technology*, 175, 594-601.

Wu, Q., Xia, X., Li, X., & Mou, X. (2014). Impacts of meteorological variations on urban lake water quality: a sensitivity analysis for 12 urban lakes with different trophic states. *Aquatic Sciences*, 76(3), 339-351.

Wu, S., Kuschk, P., Brix, H., Vymazal, J., & Dong, R. (2014). Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. *Water Research*, 57, 40-55.

Xagoraraki, I., Yin, Z., & Svambayev, Z. (2014). Fate of viruses in water systems. *Journal of Environmental Engineering*, 140(7), 04014020.

Xu, J., Zhao, G., Huang, X., Guo, H., & Liu, W. (2017). Use of horizontal subsurface flow constructed wetlands to treat reverse osmosis concentrate of rolling wastewater. *International Journal of Phytoremediation*, 19(3), 262-269.

Yan, Y., & Xu, J. (2014). Improving winter performance of constructed wetlands for wastewater treatment in northern China: a review. *Wetlands*, 34(2), 243-253.

Yildiz, F. H., & Visick, K. L. (2009). Vibrio biofilms: so much the same yet so different. *Trends in Microbiology,* 17(3), 109-118.

Zhang, C., Zhang, W., Huang, Y., & Gao, X. (2017). Analysing the correlations of long-term seasonal water quality parameters, suspended solids and total dissolved solids in a shallow reservoir with meteorological factors. *Environmental Science and Pollution Research*, *24*(7), 6746-6756.

Zhang, D. Q., Jinadasa, K. B. S. N., Gersberg, R. M., Liu, Y., Tan, S. K., & Ng, W. J. (2015). Application of constructed wetlands for wastewater treatment in tropical and subtropical regions (2000–2013). *Journal of Environmental Sciences,* 30, 30-46.

Zhang, D. Q., Tan, S. K., Gersberg, R. M., Zhu, J., Sadreddini, S., & Li, Y. (2012). Nutrient removal in tropical subsurface flow constructed wetlands under batch and continuous flow conditions. *Journal of Environmental Management*, 96(1), 1-6.

Zhang, D., Gersberg, R. M., Ng, W. J., & Tan, S. K. (2014). Removal of pharmaceuticals and personal care products in aquatic plant-based systems: a review. *Environmental Pollution*, *184*, 620-639.

Zhang, J., Chua, H. C., Zhou, J., & Fane, A. G. (2006). Factors affecting the membrane performance in submerged membrane bioreactors. *Journal of Membrane Science*, 284(1), 54-66.

Zhou, Q., Zhu, H., Bañuelos, G., Yan, B., Liang, Y., Yu, J., & Li, H. (2017). Impacts of vegetation and temperature on the treatment of domestic sewage in constructed wetlands incorporated with ferric-carbon micro-electrolysis material. *International Journal of Phytoremediation*, *19*(10), 915-924.

ANNEXURE-I

DNA Extraction Procedure

- **Lysate preparation**

  Cell pellet were prepared by harvesting up to 2×10^9 cells. Cell pellet was suspended in 180 µl genomic digestion buffer and 20 µL of proteinase K was added followed by brief vortexing. Tubes were incubated at 55° C for one hour with occasional vortexing. 20 µl of RNAse was added followed by brief vortexing. 200µl of genomic lysis buffer was added to yield a homogenous solution. 200 µl of pure ethanol was added and vortexed well for 5 seconds to get the homogenous solution.

- **Binding of DNA to the column**

  Approximately 650 µl of prepared lysate was added in spin column supplied with the kit and was centrifuged at 10000 rpm for 1 minute. Collection tubes were discarded and spin column was placed into the pure collection tube supplied with the kit.

- **Column wash**

  500 µl of wash buffer I was added in the column and was centrifuged at 10000 rpm for one minute at room temperature. Collection tube was discarded and was replaced with pure collection tube from the column. 500 µl of wash buffer II was added in the spin column and was centrifuged at maximum speed for 3 minutes at 14000 rpm. Collection tube was discarded.

- **Elution DNA**

  Collection tube was placed in 1.5 mL micro centrifuge tube. 50 µl genomic elution buffer was added in the column and was centrifuge at 10000 rpm for 1 minute. Spin column was discarded and the tube contains the pure DNA.

- **Storing of DNA**

  Purified DNA was stored at -20° C for use in PCR process.

ANNEXURE II

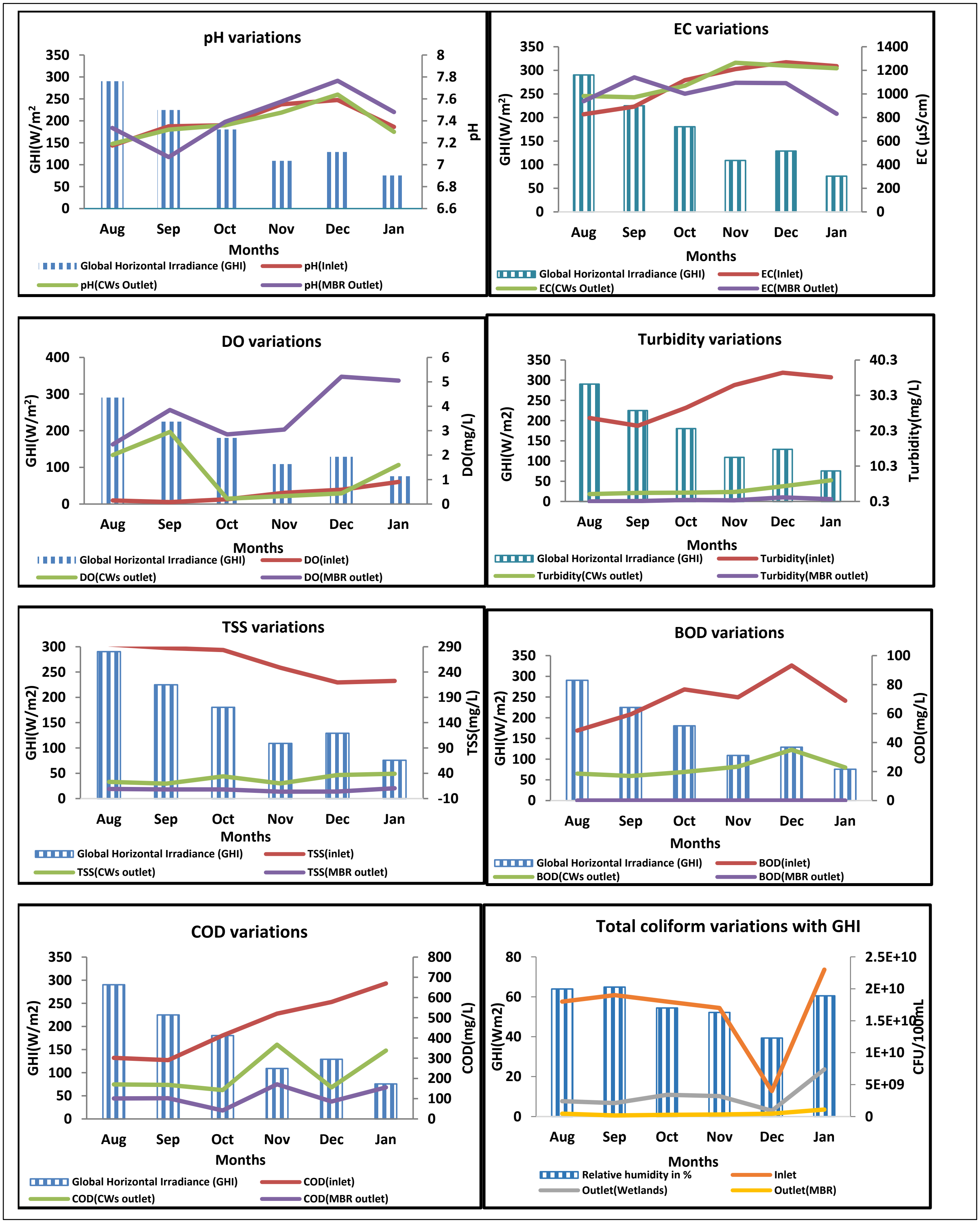


Figure 4.1: *Variations in Water Quality Parameters with Global Horizontal Irradiance (GHI)*

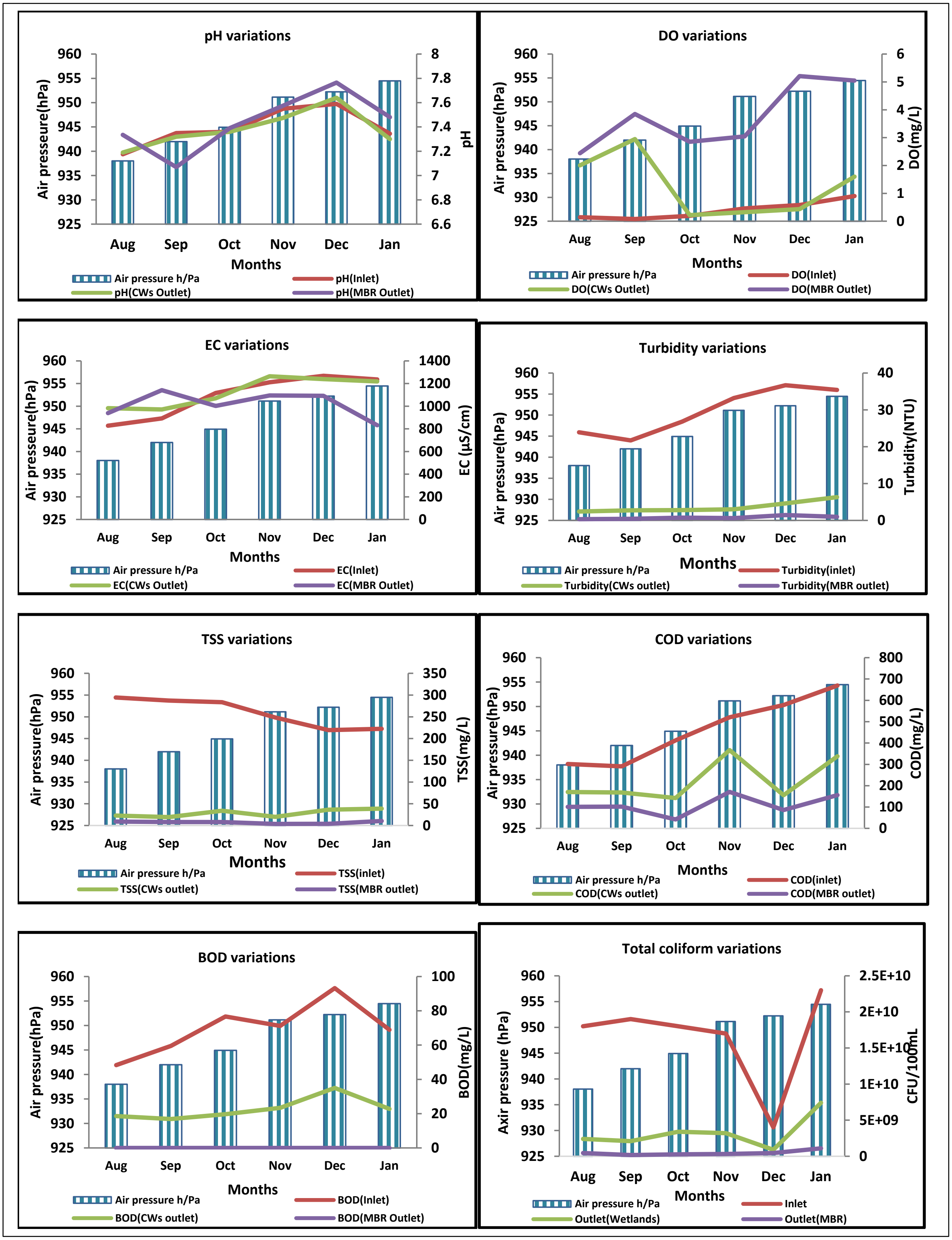


Figure 4.2: *Variations in Water Quality Parameters with Air Pressure*

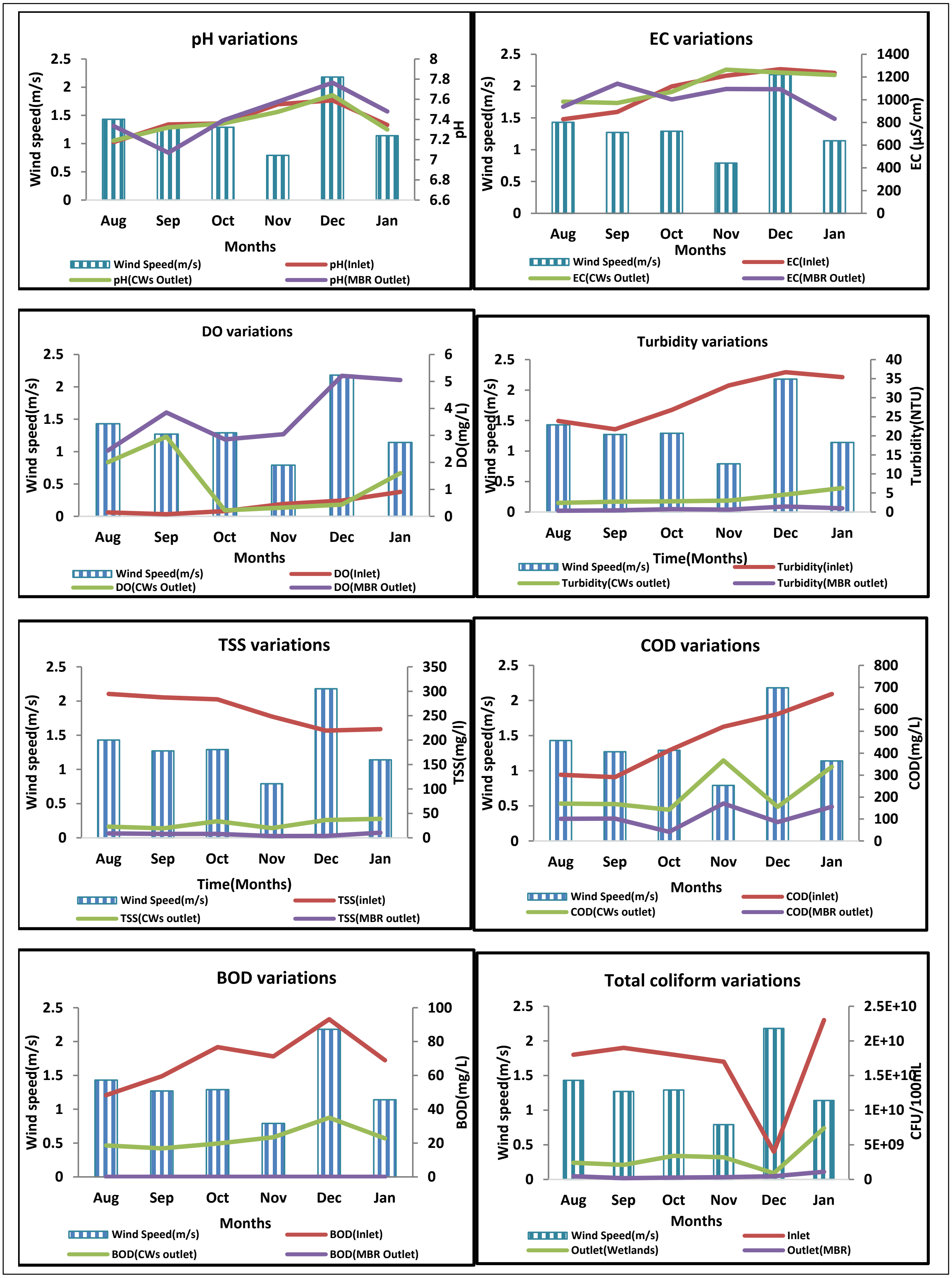


Figure 4.3: *Variations in Water Quality Parameters with Wind Speed*

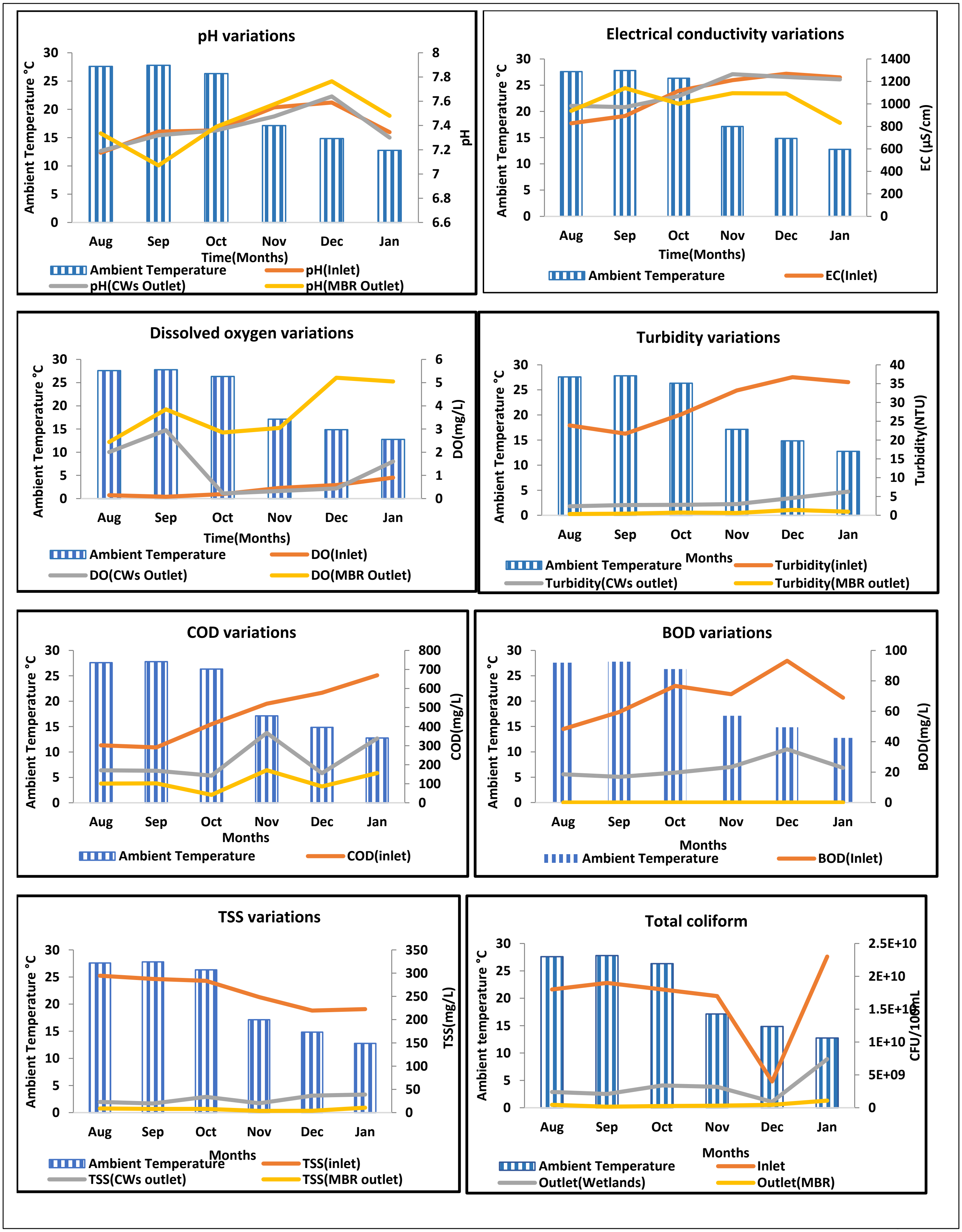


Figure 4.4: *Variations in Water Quality Parameters with Ambient Temperature*

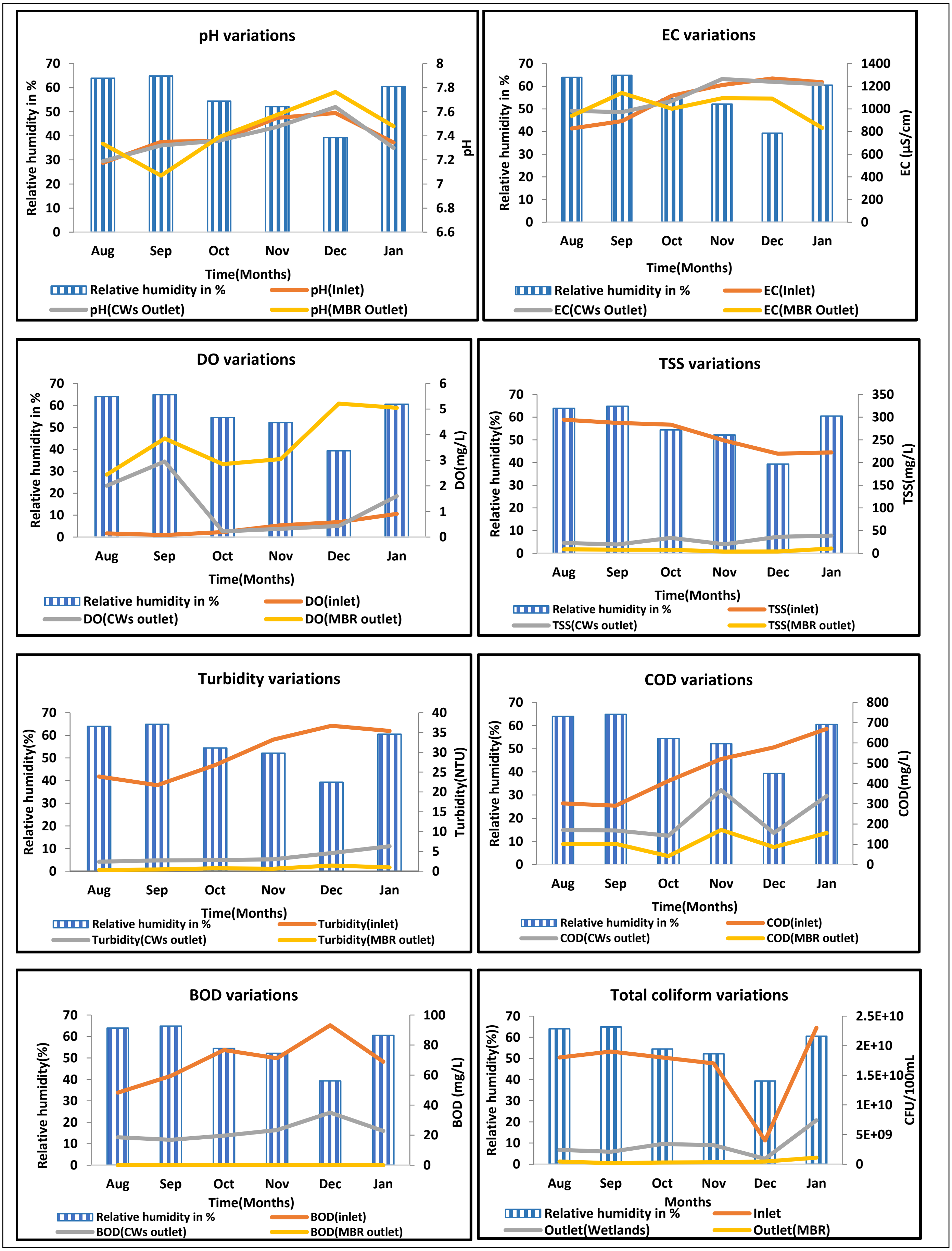


Figure 4.5: *Variations in Water Quality Parameters with Relative Humidity*