PERFORMANCE EVALUATION OF CONSTRUCTED WETLAND AND REMOVAL OF HELMINTHES PARASITES

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This thesis is dedicated to my Parents.

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Sidra Butt

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LIST OF ABBREVIATIONS

BOD	Biological Oxygen Demand
CFU	Colony Forming Unit
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DO	Dissolved Oxygen
EC	Electrical Conductivity
EMB	Eosin Methylene Blue
GHI	Global Horizontal Irradiance
MBR	Membrane Bioreactor
mg/L	Milligram per Liter
TSS	Total Suspended Solids
µS/cm	Microsiemens per cm

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ABSTRACT

Pakistan has moved from water surplus country to water scarce country. Increase in climate and environmental issues have worsened water crisis. There is a need for an effective water management and effective water technology. Constructed wetland is an engineered biological system, highly cost effective, sustainable and ecofriendly. The present study was conducted at constructed wetland located at NUST H-12 campus, Islamabad. The main aim of the study was to analyze performance efficiency of constructed wetland and helminth parasites removal along with removal of trace metals from construct wetland. In this study, continuous monitoring of different physicochemical and biological parameters was conducted from January to May 2018 and it was further statistically analyzed in relation to air temperature, global horizontal irradiance and relative humidity. Significant correlation was found between dissolved oxygen, pH, electric conductivity, turbidity, chemical oxygen demand and biological oxygen demand with air temperature, global horizontal irradiance and relative humidity (%). Removal efficiency of constructed wetland was 80 for total Suspended solids, 89 for Total *coliforms*, 70 for chemical oxygen demand, 61.2 for biological oxygen demand, and 75% for turbidity. High concentration of helminth parasite eggs was found in wastewater. Most predominant helminths species found were *Ascaris lumbricoides, Hook worms, Trichuris trichuria, Hymenolepis nana* and *Enterobius vermicularis.* Up to 100% removal of helminths eggs was observed in the wetland, which shows removal efficiency of wetland. A strong relationship was observed between selective meteorological and water quality parameters (Global horizontal irradiance, relative humidity, total *coliforms*, ambient temperature, turbidity, and total suspended solids) and helminths eggs concentration. Removal efficiency of Constructed wetland was 53, 80, 70 and 71% for Zinc, Lead, Manganese, and Iron. Highest uptake of trace metals was observed in *Typha latifolia* due to its high biomass and efficient root system. Highest concentration of iron uptake from all wetland plants was observed during July. Highest concentration of metals was observed in roots than leaves and shoots. Results shows that performance efficiency of constructed wetland is highly influenced by seasonal variation. It also depicts that constructed wetland is capable for improving water quality.

Key words: *Constructed wetland, Physico-chemical parameters, Biological parameters, Meteorological parameters, Biological treatment system*

1. Introduction

1.1 Background:

Water is major element of all forms of life existing on earth. It covers up to 70% of total earth's surface out of which only 2-3% is fresh water. There are two major sources of water i.e. ground water and surface water which mainly includes fresh water lakes, streams and rivers (Khan *et al.,* 2018).

Water scarcity is becoming a major global issue because of rapid increase in population. It is resulting stress in the available water resources and leading toward deterioration of water quality by mixing of untreated wastewater with fresh water bodies. Also, resulting in exploitation of natural water resources to meet their water demand (Zhang *et al.,* 2014).

1.2 Wastewater and its type:

Water pollution is defined as impairment of water quality by addition of wastewater and harmful contaminant in water bodies and it is unable to support human life. This pollution results in adverse changes in water quality and harm water dependent aquatic life. There are two major categories of water pollution i.e. direct discharge of factories and waste treatment plants and refineries and indirect addition of contaminants by atmosphere, ground water systems and soils systems including agriculture runoff containing pesticides and fertilizers (Bhatia and Goyal, 2014).

Discharge of untreated wastewater results in harmful ecological, contamination of sanitation supplies and drinking water supply health impacts. According to Environmental Protection Agency, wastewater should be treated before its discharge in to fresh water bodies to prevent contamination of surface water and adverse soil contamination. Untreated wastewater results in accumulation of organic matters, pollutants and nutrients leading to oxygen depletion and eutrophication in aquatic ecosystem (Islam *et al.,* 2016).

There are mainly four types of wastewater including domestic, agricultural, storm water and industrial wastewater. Agricultural wastewater mainly consists of water generated from agricultural sector i.e. pesticides and fertilizers runoff. Whereas urban wastewater is combination of domestic and agricultural wastewater including storm water and sewage infiltration (Mousavi *et al.,* 2015). Characterization of wastewater is mainly done based on source and its effects.

Table 1.1: Types of wastewater

Types of wastewater	Sources	Effects
Industrial Wastewater	Commercial and industrial activities	Chemical reactions with environment due to presence of harmful chemicals which result in depletion of oxygen
Storm wastewater	Mainly includes rain runoff from urban and agricultural areas into drains	It results in harmful effect of aquatic life. And make waterways unhealthy place to play, live and work
Domestic wastewater	It mainly comprises household and domestic wastewater	It contains disease causing pathogens i.e. cholera or typhoid

1.3 Water situation in Pakistan:

In Pakistan with every year demand for water resources is rapidly increasing as increase in population. The population of Pakistan is anticipated to be double by the year 2025. Pakistan has moved from water stressed country to water scarce country. It is observed that significant fall in per capita water availability in Pakistan resulted in shortage of water. If trend remains same, Pakistan will also face extreme water scarcity by 2025 (Briscoe *et al.,* 2005). It has led to great stress on fresh water available resources and management of untreated wastewater. It is projected that water availability per capita will be less than 700 m^3 by the end of 2025. Increase in climate change, political instability and uncapable waste management has worsened water crisis which is rising conflicts among people for availability of water which is basic right (Murtaza and Zia, 2012). Recent study conducted in 2015 has showed decrease in per capita annual water availability of Pakistan from 5,000 m^3 in 1951 to 1,038 m^3 in 2010 which is close to International water scarcity level. Report by IMG has designated Pakistan in top water stressed country with availability of water by 1,017m3 per capita. (Haydar *et al.,* 2015). Poor management of industrial, domestic and agricultural wastewater has resulted in emergence of serious health concerns, fresh water bodies contamination and reduction in agricultural productivity. Direct release of wastewater in waterbodies not only deteriorate water quality but also result in water borne diseases. Depending upon source of wastewater, it contains disease causing pathogens (viruses, bacteria and parasites), highly toxic metals, excessive nutrients and pesticides.

1.4 Solution to wastewater treatment:

To resolve water crisis there's a need for an effective strategies of water management and strict implementations and monitoring. There are number of different wastewater treatment technologies to cope up with waste issue. Most of such technologies are beyond the reach of developing countries because of high cost and poor implementation of protocols. Little or no importance is given to waste treatment systems in poor countries which results in release of untreated wastewater in to streams and lakes. (Wang *et al.*, 2018).

1.5 Constructed wetland is an example of biological systems in which plants are used to remediate or clean wastewater. It is an engineered novel solution for wastewater treatment which is cost and energy effective sustainable and eco-friendly. It has three sections including untreated wastewater at inlet, vegetation and treated wastewater outlet. Vegetation is an important section of constructed wetland which contains potential plants with ability to accumulate high concentration of compounds. It is highly due to its great potential for improving water quality and effective nutrient and efficient for removal of biological nutrients. hazardous substances removal, it is gaining high importance throughout the world (Birkigt *et al.*, 2018). It includes interaction of microbes, substrates and vegetation in a controlled environment. It is considered as a best option for treatment of domestic, municipal and gray wastewater. Constructed wetland is considered as affordable tool for wastewater treatment and reclamation in semi-arid and arid areas (Vymazal, 2011). Due to its simple operation and low maintenance and operational cost, it is an efficient technology for developing countries to improve quality of water (Zurita *et al.*, 2011).

1.6 Present study

This study was conducted to evaluate performance efficiency of pilot scale constructed wetland with seasonal variation. It also aims at determination of helminth eggs in wastewater and also evaluate removal of these helminths by constructed wetland. Further trace metals determination of wetland plants was determined, and heavy metal uptake was analyzed.

1.7 Objectives

1. Monitoring of performance efficiency of constructed wetland by various physicochemical and biological parameters
2. Estimating a correlation between meteorological parameters and water quality parameters
3. To determine the Helminth parasites in treated and raw wastewater in Constructed Wetland
4. Evaluate concentrations of metals in Constructed Wetland to provide an insight into the current pollution status

2. Literature Review

2.1 Water Availability:

Up to 85% of world population is living in water scarce part of earth. Rapid urbanization and continuous industrialization have resulted in limited availability of natural resources mainly Fresh water resources. Global increase in population has resulted in rapid decline in availability of fresh water. Non-availability or limited access of water has resulted in need for new resources to fulfil water demand. With the emergence of Sustainability concept in 1992, a lot of different concepts are being introduced including water recycling, reuse and efficient use of water. Financial and social constrains limits developing countries in implementing proper sanitation and water infrastructures (Woltersdorf *et al.*, 2018). According to WHO report up to 2.4 billion people in developing countries lack basic sanitation and wastewater treatment facility (WHO, 2016). Up to 70 % of total freshwater is consumed by the agriculture sector alone which make it a major cause of decline in freshwater.

2.1.1 Wastewater Treatment Overview:

Depending upon type and quantity of wastewater, there are number of different methods being used including biological, physical and chemical for wastewater treatment.

2.1.1.1 Physical Methods:

In this system, physical process and methods are being used to treat and enhance quality of wastewater. Major physical process included sedimentation, screening and filtration of coarse particles. Sedimentation is one of basic method which is mainly used at beginning of wastewater treatment process to eliminate solid particles from water. In this process, settling of solid particles is mainly done by gravity over a period. One of other important physical method is filtration in which wastewater is allowed to pass through filtrates of different porosity to remove solid particles. Air dissemination is also an important method in which air in introduced in wastewater. Physical methods also include membrane filtration and deep filtration beds (Zarei *et al.*, 2018).

2.1.1.2 Chemical Methods:

Chemical methods of wastewater treatment include introduction of different substances to improve water quality. Chlorination is one of important chemical method in which chlorine compound is used to remove microorganisms and improve water quality. Bacterial removal is significantly enhanced in the presence of chlorine. (Hu *et al*, 2018). Coagulation included usage of different coagulants which combines with wastewater and it can be easily separated from water. Different metals i.e. Alum, different iron blends and lime is commonly used coagulants. Ozone is also used as an important oxidizing disinfectant, to enhance water quality. It is mainly used in potable and non-potable water. Its high oxidizing power make it best choice for disinfection and oxidation. It is preferred over other chemical alternatives because it is readily available and is rapidly decompose in to oxygen. It eliminate color and wastewater odor (Bourgin *et al.,* 2018).

2.1.1.3 Biological Methods:

Different biological methods are being used for effective nutrient and organic matter removal. Phytoremediation emerged as promising biological method in which different plants are used to remove excess nutrients and contaminants from aquatic ecosystem. This remediation technology includes usage of different green plants for removal of nutrients from wastewater and soils. Mainly photosynthetic plants are used to phytoremediation process. Constructed wetlands are biologically enhanced engineered structure which are designed to treat wastewater. Constructed wetlands are also used to screen phytoremediation potential of green plants and determine their effectiveness for maintain physicochemical properties of wastewater within range. Also, to closely monitor rate of transformation of organic and inorganic contaminants.

2.2 Constructed Wetland:

Constructed wetlands are considered highly economically feasible for reuse of effluent keeping financial constraints in account. Constructed Wetlands are involved in effective removal of variety of pathogens including intestinal parasites mainly from wastewater (Amahmid *et al.,*2018). Constructed wetlands are biologically enhanced engineered structure which are designed to treat wastewater. Constructed wetlands are also used to screen phytoremediation potential of green plants and determine their effectiveness for maintain physicochemical properties of wastewater within range. Also, to closely monitor rate of transformation of organic and inorganic contaminants (Sricoth *et al.,* 2018).

2.2.1 Overview:

This system mimics natural wetlands. It is considered as novel and environmentally friendly technology for wastewater treatment. Different types of wetlands include subsurface flow, free water surface flow and hybrid wetlands. Selection of suitable constructed wetland depend mainly on type of pollutants to be removed, economic feasibility, availability of land, and geography of the location. Constructed wetlands included different chemical, biological and physical process for nutrient removal and improving quality of water. For developing countries, it is considered as best sustainable way of wastewater treatment, which is cost effective, cheap and reliable (Aydin Temel *et al.,* 2018). For developing countries constructed wetland is considered as a best alternative treatment system because of its beneficial economical, ecological and environmental characteristics. It is preferred over conventional wastewater treatment methods because it is simple to operate, low operational and maintenance cost, low investment cost and provide high quality effluent. Also, removal efficiency of Chemical oxygen demand, suspended solids, pathogens removal and Biological oxygen demand is higher for improving water quality (Alufasi, 2017).

2.2.2 Historical Development:

Concept of construed wetland is not new for wastewater treatment. Wetlands were emerged naturally due to frequent discharge of water in natural systems. From a long time, g, wetlands are being used for treatment of water discharged i.e. sewage. In old times ancient Chinese and Egyptians used natural wetlands for wastewater disposal. Oldest documentation of constructed wetland was first noted in 1904 in NSW, Australia for the treatment of residential wastewater (Arheimer *et al.,* 2017).

2.2.3 Constructed Wetland and its functioning:

Wetland is specific type of ecosystem which is identified by its distinguishable soil and water conditions. Wetland soil is mainly saturated with water or poorly aerated. This ecosystem mainly supports hydrophytes which easily survive in wet conditions (Ma *et al.,* 2018). Construed wetlands are engineered structures which is also known as manmade or artificial wetlands. These wetlands operate in controlled environment by utilizing microbial communities, vegetation and soils. Wetland microorganisms have great potential to degrade organic material from wastewater. These microorganisms attached with wetland media i.e. gravels, roots of plants, organic matter, synthetic material (Wang *et al.,* 2018). Another study by Doherty and his coworkers in 2012 showed significant and considerable growth of microbial communities in planted constructed wetlands as compared to unplanted structures. However, there is need for more research to show effect of plants growth on microbial activity.

2.2.4. Pollutant removal process in constructed wetland:

Number of basic procedures are helping in improving quality of wastewater in CWs. These procedures include screening, sedimentation, adsorption, precipitation, filtration, and microbial activities. Process of constructed wetland can be easily demonstrated by following diagram below.

2.3 Types of Constructed Wetland

Following are types of constructed wetland based on water column level listed below:

1. Free water surface treatment wetland

2. Sub- surface flow wetland

Further, constructed wetlands are also classified on the type of vegetation used in wetland i.e.

1. Submerged macrophytes wetland
2. Floating macrophytes wetland
3. Root emergent macrophytes wetland

2.3.1 Free water surface treatment wetlands:

FWS treatment wetlands resembles natural wetlands. In such wetlands, wastewater flows over the soil surface. These wetlands have higher net carbon production because of high primary production and low rate of decomposition in oxygen limited water column. It is highly effective for significant removal of pollutants because of autotrophic process of wetland. It is cost effective and can treat huge volumes of wastewater. FWS are lined with impermeable membranes to avoid any infiltration of contaminated water in soil. Such wetlands are more effecting in treating farmyard runoff. It shows highest total solids removal, and moderate removal of pathogens. It is more suitable for small areas where pretreatment facilities are already available. Water layer is aerobic whereas deep layers are anaerobic. Such wetlands are highly effective for removal of organic microcontaminants.

2.3.2 Subsurface flow constructed wetlands:

Subsurface flow constructed wetlands are getting more attention in developing countries because of its common system and ecotechnology. Treated water from these wetlands showed decrease in Biological oxygen demand and total suspended solids. Usually sand and gravel are used as a porous media and it should be able to support emergent plants. Such wetlands should be developed in such way that water level should be always lower than top level of substrate. These wetlands are further divided in to two types i.e. Vertical subsurface flow and horizontal subsurface flow. In vertical flow constructed wetland, wastewater is fed in large batches which is allowed to pass through beds and over the surface toward the drainage network. In horizontal flow constructed wetland, water is fed through inlet and water is allowed to pass through porous medium under emergent plants.

2.4 Type of Vegetation

Large aquatic macrophytes including algae, mosses and vascular plants are used for phytoremediation process. These Macrophytes are very significant for wetland design and also in treatment of wastewater; Wetlands are mainly differentiated from other lagoons and sand filters due to the presence of number of macrophytes. Another study by Aziz and his coworkers showed that Wetland plants have very rich roots and rhizomes which help them to collect excessive nutrients and contaminants and highly tolerant to high organic loadings by acting as a reservoir for microorganisms and helping in water purification. High pathogen removal by macrophytes is due to the presence of antibacterial properties of plants and also specialized rhizomes. (Aziz *et al.*, 2015). Planted wetlands show effective removal of nutrients and pollutants as compared to unplanted wetlands. Březinová & Vymazal studied growth rate of macrophytes in constructed wetland and study showed that Highly defined rhizomes and roots provides microorganisms large surface area for growth and reducing carbon and oxygen content. For selection of suitable plant species for wetland, growth rate, biomass and below ground organic should be considered (Březinová & Vymazal,2015).

2.5 Physicochemical properties of constructed wetland:

Constructed wetlands play a very crucial role in improving quality of water and removal of nutrients (Liu *et al*., 2018). The water quality parameters include physical, biological and chemical parameters mainly pH, Turbidity, Total Suspended Solids, Total Dissolved Solids, Electric Conductivity, Chemical Oxygen Demand, Biological Oxygen Demand, Dissolved Oxygen and Temperature (Huang *et al.*, 2015).

2.5.1 Dissolved Oxygen:

Dissolved Oxygen refers to Oxygen gas dissolved in water. Presence of aquatic species is dependent upon abundance of dissolved oxygen concentrations. Photosynthesis is main source of Dissolved Oxygen in water bodies (Mukherjee *et al.*, 2018). Decline in Dissolved Oxygen is due to mixing on sewerage water from household and industrial sector. Microbes use Dissolved oxygen to degrade/decompose organic material present in wastewater, which lead to decline in dissolved Oxygen concentration in water. Low concentration of Dissolved Oxygen can lead to decline or complete elimination in species diversity.

2.5.2 pH

pH is defined as acidity or basicity of an aqueous solution. A scale ranging from 0-14 where 7 is considered as neutral, 0-6 acidic and about 7 is considered as basic. Water within range of 6.5-9 is considered suitable for reuse purpose. High concentration of cations results in acidity whereas high concentration of anions results in basicity.

2.5.3 Total Dissolved Solids:

It constitutes mainly organic and inorganic salts dissolved in water including magnesium, calcium and sodium cations and bicarbonates, carbonates, sulphates and chlorides anions.

2.5.4 Turbidity:

It is defined as optical property of waste where light is scattered due to the presence of suspended and dissolved materials and microscopic organisms. Turbidity provides an estimate of amount of Sediments in water sample. Turbidity is measured by the relative scattering of light beam through a restricted range of angles. Usually light beam by water sample scattered at 90^0 (Skoronski *et al.*, 2018).

2.5.5 Total Suspended Solids:

Total suspended Solids represents actual amount of organic particles and minerals transported in water column. It is linked with nutrients, industrial and agricultural chemicals and metals transport. It mainly contains sediments consisting of silt and clay particles that are easily transported and easily deposited. It is measured by residue of water sample which do not pass through glass fiber filter. The trapped residue is further dried and reported in mg/L units (De Robertis *et al.*, 2003).

2.5.6 Chemical Oxygen Demand:

It is measure of capacity of water body to consume oxygen during oxidation of inorganic chemicals and decomposition of organic matter. It is widely used for monitoring, modeling, designing and operational analysis of plants. It is measured by conventional closed reflux titration method using dichromate reagent and sulphuric acid. It can be also defined as number of oxygen equivalents required for oxidation of organic pollutants in water (Dubber & Gray, 2010).

2.5.7 Biological Oxygen Demand:

Biological Oxygen Demand is considered as an indicator of organic contamination of river. It is defined as amount of oxygen required by microorganisms for their respiratory activity in order to degrade organic compounds incubated at 20^0C for 5 days (Jouanneau *et al.*, 2014). Biological Oxygen Demand is measure of amount of organic pollutant that can be easily degraded by biological means (Xiao *et al.*, 2015).

2.5.8 Electric Conductivity:

It reflects capacity of water to conduct electricity. Electrical Conductivity is dependent upon concentration of dissolved salts in water. It is measured as Siemens per meter [S/m] in SI and millimhos per centimeter [mmho/cm]. High Electric Conductivity indicates high concentration of conductive ions in water. Conductive ions i.e. carbonate, sulfides, chlorides and alkalis compounds. Electric Conductivity of water increases with increase in temperature (Rashid *et al.*, 2015).

2.5.9 Total Coliforms:

Coliforms are group of microorganisms defined as gram negative, rod shaped bacteria which has ability to ferment lactose and release gas when incubated for 24 hours at 35-37^0C Optimum temperature. It is an important indicator for sanitary quality of water which cause multiple diseases in animals and humans (Gruber *et al.*, 2014).

2.6 Correlation of physicochemical and biological parameters with meteorological parameters:

Different studies have been conducted to determine significant impact of meteorological parameters on water quality parameters.

In 2017, Zhou and his coworker carried out a study to investigate effect of temperature on performance of constructed wetland. Study showed a substantial impact of temperature on constructed wetland (Zhou *et al.*, 2017)

A study was conducted by Ahsan and his coworkers in which effect of temperature on wastewater treatment was studied. The study evaluated the effect of temperature on removal of suspended solids and Chemical Oxygen Demand. The result showed a significant enhancement of removal of Suspended Solids and Chemical Oxygen Demand by increasing temperature (Ahsan *et al.*, 2005).

Significant correlation was found between multiple water parameters i.e. BOD, COD, Total Solids, Electric Conductivity, Total Phosphorus. This study was carried out in China by Zhang and his team in 2017. Regression model was used to study correlation between water quality parameters and meteorological parameters (Zhang *et al.*, 2017)

Similar study was reported by Major and his coworker in 2017, a study was carried out in kettle ponds in Poland to study impacts of weather variation on physicochemical parameters of water. It was observed that meteorological variation causes significant impact on ions concentration of water body, these variations are mainly due to vertical exchange of air (Major & Cieśliński, 2017).

Another research was conducted in Egypt to study and measure seasonal variation of water quality parameters i.e. Dissolved Oxygen, Electric Conductivity, Total Dissolved Solids and pH in relation to meteorological parameters i.e. relative humidity and temperature. Results showed a significant positive relation between humidity and temperature and electric conductivity, pH and total dissolved solids. Whereas, there was a negative correlation with Dissolved Oxygen, and no significant relation of turbidity was noticed (Sallam & Elsayed, 2018).

2.7 Microbiological guidelines for Wastewater:

Wastewater act as a vehicle for transmission of biological infections, especially viruses, Protozoa and bacteria. These infections agents can be easily transmitted by direct contact with wastewater or either indirect contact by ingestion of infectious crops (Khallayoun *et al.*, 2009). These parasites use human body as a source of host for them and cause different diseases in human. Low health risk is associated with bacteria as compared to helminths because of their parasitic nature. Helminthic parasites are highly resistant to environmental stress and can easily persist up too many years. The persistent ability of helminths is big question mark for the reuse of wastewater in agriculture sector (Chaoua *et al.*,2018). To counter this issue, WHO has developed guidelines for permissible level of parasites in water and removal of helminths eggs from wastewater. Following guidelines is categorized depending upon reuse condition and number of helminths eggs per liter of wastewater.

Table 2.1: Health guidelines for the use of wastewater in agriculture source (WHO, 2017)

Category	Reuse Conditions	Exposed Groups	Intestinal Nematodes (Arithmetic mean no. of eggs per liter	Faecal Coliforms (Geometric mean no. per 100 mL

A	Irrigation of crops, likely to be eaten uncooked. Sport fields and public parks	Workers, Consumers, Public	≤1	≤1000
B	Irrigation of cereal crops, fodder crops, industrial crops, tress and pastures	Workers	≤1	No standard recommended
C	Localized irrigation of crops in category B if exposure of workers and public doesn't occur	None	Not applicable	Not applicable

For effective removal of helminths eggs, Engelberg Indicator should be met: number of helminth eggs per liter< 1 (Motevalli *et al.*, 2015). Nematode eggs mainly helminth eggs are slightly heavier and bigger than other protozoan cysts, which allows them to settle out more easily and effectively. Constructed wetlands are highly effective for removal of helminth eggs from wastewater and reduction up to 99.9%. Mainly present nematodes in wastewater are *Ascaris lumbricoides*, *Hymenolepis nana* and *Trichuris trichuria* (Santos *et al.*, 2014).

High number of helminth eggs mainly *Ascaris lumbricoides* and *Hymenolepis nana* were found in raw wastewater. To reduce parasite infections in environment, public must be encouraging to ensure proper usage of disinfectants and better wastewater treatment system (Sharafi *et al.*, 2015).

Another study conducted by Abagale and his worker in 2013 revealed that helminth eggs type and diversity is very important to look at seasonal distribution and it is highly influenced by environmental factors i.e. temperature, sunshine amount, rainfall and humidity. High species diversity is mainly observed during favorable low temperature and high humidity wet season as compared to dry season (Abagale *et al.*, 2013). *Ascaris lumbricoides* is the most frequent and prevalent in raw wastewater. High resistant to external conditions allows it to remain viable for longer timer period. Presence of *Ascaris lumbiocoides* is mainly correlated with poor socioeconomic condition and public health standards (Mahvi and Kia, 2006).

Similar study was conducted by Stott and his coworker Mara, to enumerate parasite eggs and analyze effect of different physicochemical parameters on helminth eggs removal efficiency. Field scale and pilot scale ponds and wetlands were studied for removal of helminth eggs from wastewater. Up to 99.9% parasite eggs were reduced (Stott and Mara, 2003).

2.8 Heavy metals accumulation in Constructed Wetlands:

Macrophytes are very important for accumulation of trace metals in plants tissue and promoting variety of chemical and biological processes. Constructed wetlands are very effective with high trace metals removal efficiency. Several studies have been conducted to investigate accumulation of trace metals in organs of macrophytes. Greater Biomass play an important role in high elemental concentration. Macrophytes have great inclination toward elements phytostabilization. Also, macrophytes with highly developed roots system are considered most suitable for elements accumulation (Bonanno and Vymazal, 2017).

In a study conducted in 2015, Constructed wetlands were evaluated for mass removal and heavy metals removal including lead, arsenic, manganese, vanadium and boron. High removal efficiency was observed for Iron (44%), Cobalt (31%) and Boron (40%). Trace metals accumulation process is highly dependent upon sediments which play an important role in accumulation and adsorption of pollutants. Also, plants with well-established root system act as phytostabilizers (Morari *et al.*, 2015).

Similar study was conducted by Klink to investigate ability of trace metal accumulation and potential use of *Typha latifolia* and Phragmites *australis* in phytoremediation of elemental pollution. The study showed high correlation of *Phragmites* leaves with high Fe, Mn and Cd concentration. Whereas, High Zn, Cu and Pb concentration was observed in *Typha latifolia* leaves. Study showed following accumulation trend for Cu, Ni, Pb, Fe and Zn concentration: roots> rhizomes> leaves> stems. Amount of metal accumulation was also observed high during vegetation process (Klink, 2017).

To evaluate and monitor heavy metal concentration in a constructed wetland, plants and sediments samples were collected from and further analyzed for trace metals concentration. Concentration of Zn, Pb, Cd, and Cu in sediments was above the Dutch intervention guidelines. Wetland Plants should be replaced after long term operation as accumulation of heavy metals in roots increase with passage of time and it further cause adverse impacts on plants functioning (Liang *et al.,* 2018).

Keeping future perspective of constructed wetland, enhancement of heavy metals uptake is still in progress and a lot of work need to be done to further enhance it.

3.Material and Methods

In this chapter, details of methodology adopted has been discussed briefly.

3.1 Study Site:

The biological wastewater treatment system studied is located at National University of Sciences and Technology, H-12 Islamabad with following coordinates.

Length: 73 ° 00'13.7 "E   Latitude 33 ° 38'31.1 "N

Meteorological parameters play a very crucial role in efficient working of constructed wetland. Macrophytes and microbes are mainly dependent on temperature.

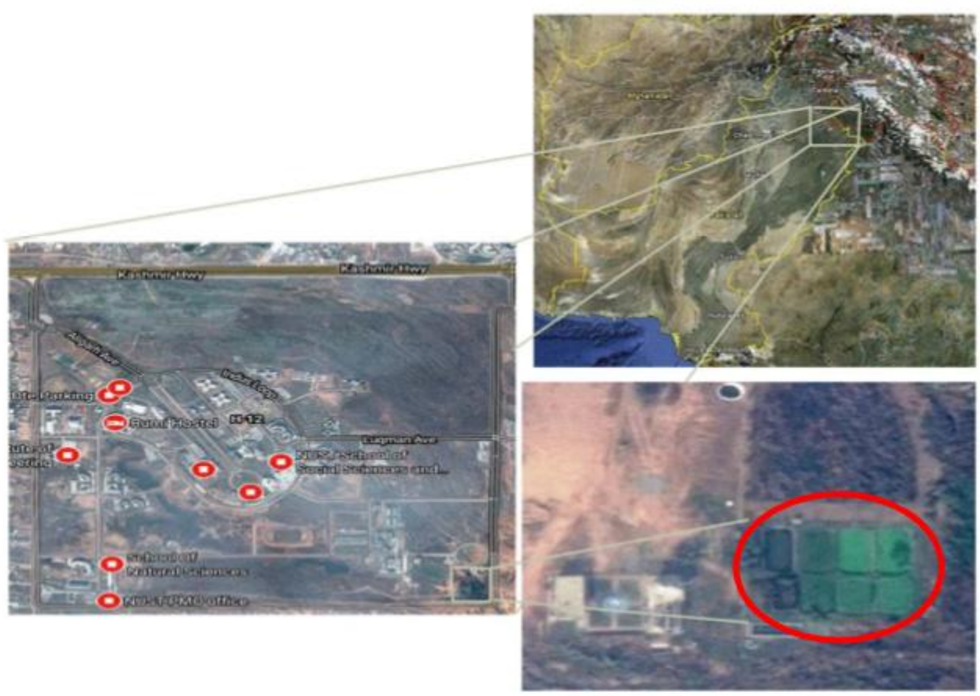

Figure 3.1 Geographical location of NUST constructed wetland

3.2 Description of Constructed Wetland:

This treatment system is funded by USESCO as technology demonstration for wastewater treatment. It is used for the treatment of wastewater from the residential area and academia including departments and hostels. On average 200,00 US gallons per day is generated by population of 6000. Up to 25000 US gallon per day is maintained by the constructed wetland. Construed wetland can treat 0.1 MG water per day. It is a three-stage wastewater treatment plant. A schematic diagram of wastewater treatment facility developed at NUST is shown in figure 3.2 below.

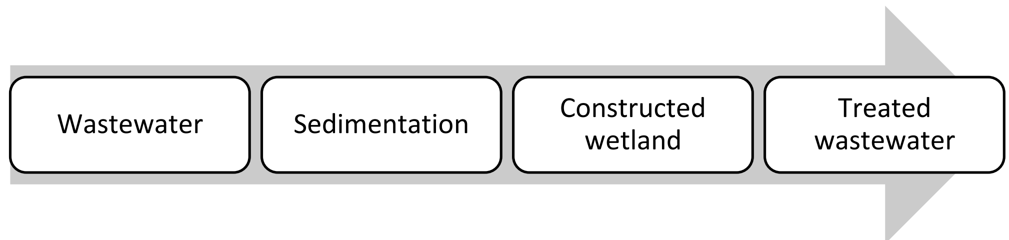

Figure 3.2 Schematic diagram of wastewater treatment facility

Flow of wastewater is checked through a rotating valve. Wastewater is first allowed to pass through sedimentation tank to pre-treat water, before entering the stated secondary treatment by constructed wetland. 18850 US gallons of wastewater is allowed to pass through sedimentation tank to pretreat it before loading in to biological treatment system. All treatment ponds are interconnected with each other. Ponds are lined with plastic sheets to avoid seepage and percolation. Four different types of plants including *Typha latifolia*, *Centella asiatica*, *Lemna gibba*, and *Pistia striotoes* have been planted. Eighth pond served as an outlet for the system.

3.3 Sampling:

Sterilized autoclaved bottles were used for sample collection. For physicochemical analysis, samples were collected weekly in a month from wastewater inlet and treated wastewater outlet of constructed wetland. Collected samples were transferred to Environmental chemistry laboratory at Institute of Environmental Sciences and Engineering (IESE) for further analysis. All sample were analyzed under the standard method for the examination of water and wastewater. (APHA,2012). All laboratory tests were performed on same day to get maximum accuracy and avoid changes on physical and chemical characteristics.

3.4 Water Quality Analysis:

3.4.1. Physico-chemical Analysis:

In Table below different parameters, instruments and methods used for analysis are listed.

Table 3.1: Characterization of water quality parameters with units

Parameter	Instrument	Units
pH	pH Meter (HACH 156)	-
Temperature	HACH session 1	˚C
Dissolved Oxygen	DO meter (HACH 156)	mg/L
Electric Conductivity	EC meter (Ino lab 720)	µS/cm
Total Dissolved Solids	Analytical Mass Balance	mg/L
Total Suspend Solids	Analytical Mass Balance	mg/L
Turbidity	Turbidity meter (HACH 2100 q)	NTU
Biological Oxygen Demand	DO meter (HACH 156)	mg/L
Chemical Oxygen Demand	Titration method	mg/L

On site analysis:

Temperature, pH was measured using HACH session 1 and HACH 156 respectively. DO meter (Crison Oxi 45) was used to measure Dissolved Oxygen concentration in water samples.

Laboratory Testing Analysis:

Parameters including Electric Conductivity, Total Dissolved Solids, Total Suspended Solids, Chemical Oxygen Demand and Biological Oxygen Demand were analyzed in laboratory within 3 hours of sample collection. Electric conductivity and Total Dissolved Solids were measured by potentiometric method using portable conductivity meter. Total suspended solids were measured through gravimetric dried method. Chemical oxygen demand was measured using close reflux method by titrating against ferrous ammonium sulfate 0.1 N.

3.4.2 Microbiological analysis:

Weekly microbial analysis of wastewater samples was performed. Total *coliforms* were analyzed along with technique and media used.

Table 3.2: Biological parameter

Parameters	**Technique Used**	**Media Used**	**Measured Units**
Total *Coliforms*	Membrane Filtration (MF)	Eosin Methylene Blue Agar	CFU/100 ml

3.4.2.1 Preparation of Media and agar plates:

Eosin methylene blue (EMB) agar was used as a media for total coliforms. Agar was prepared by weighing 37 grams of media in 1000ml of distilled water and poured on autoclaved petri plates. After pouring media plates sterility was determined by placing in incubator for 24 hours.

3.4.2.2 Dilution preparation:

All water samples were serially diluted and diluted samples were pass through membrane filter (0.45 µm size) fitted in filtration assembly. Membrane filter were placed over prepared EMB agar plates and were allowed to incubate for 24 hours at 37 C. Colonies were counted over colony counter.

3.5 Acquisition of meteorological analysis

To determine effect of meteorological parameters on water quality parameters, data was acquired from US-Pak Center for Advanced Studies in Energy. 5 months data qas acquires starting from January 2018 to May 2018.

3.6 Helminth Eggs count and identification in wastewater:

Enumeration and identification of helminth eggs in wastewater was determined using standard method of Laboratory manual of Parasitological and Bacteriological Techniques (WHO, 1996)

Raw water and treated wastewater sample of known volume was collected, and samples was further allowed to sediment for 24 hours. After sedimentation, 90 % of the supernatant was removed by pouring it off. Sediments were transferred in to centrifuge tubes depending on the volume of sample and centrifuged at 1000 g for 15 mins. Supernatant was removed, and all sediments were transferred in to one centrifuge tube and again centrifuged at 1000 g for 15 minutes. Pellet was suspended in to equal volume of acetoacetic buffer solution. 2 volumes of ethyl acetate were added, and solution was thoroughly mixed with vortex mixer. Sample were centrifuged again at 1000 g for 15 mins. Three distinguishable layers were observed in the centrifuged tube. Volume of the pellet was measured, and rest of supernatant was poured off. Pellet was again resuspended in 5 volumes of zinc sulphate solution. Using Pasteur pipette aliquot was transfer to the McMaster slide. McMaster was observed in microscope using 10X and 40Xmagnification.

Number of eggs per liter were calculated using following equation

$$N = AX/PV$$

Where

N = number of eggs per liter of sample

A = number of eggs counted in the McMaster slide or the mean of counts from two or three slides

X = volume of the final product (ml)

P = volume of the McMaster slide (0.3 ml)

V = original sample volume (liters)

3.7 Trace metals analysis in constructed wetland:

Following steps were carried out for metal analysis.

3.7.1 Sample Collection:

Water and plants samples were collected from different ponds of constructed wetland.

Water samples were stored in autoclaved bottles.

Plants samples were harvested by using stainless steel in order to reduce chances of contamination, sediments samples were placed in zip locked plastic bags. After reaching laboratory, plant samples were thoroughly washed with water and then by distilled water to remove any adhering material present there. Sample collection is done at peak time of seasonal growth. Plants and sediments samples were stored in plastic bags before further analysis is carried out. Few drops of HNO_3 were added to avoid precipitation of metals in water samples.

3.7.2 Sample Analysis:

Sample were analyzed using Atomic Absorption Spectrophotometer to determine concentration of trace metals present in water samples and plants tissue.

3.8 Statistical Analysis:

- Significant and Non-Significant correlation with significance level at $p<0.05$ was noted between meteorological parameters and water quality parameters.
- Mean of triplicate values was calculated and standard deviation was applied.

4. Results and Discussions:

4.1 Monitoring performance efficiency of Constructed Wetland by various Physicochemical and Biological Parameters:

Water samples were weekly collected from inlet and outlet points of constructed wetland for their physicochemical and biological analysis for period of 5 months starting from January 2018 to May 2018. Analyzed parameters shows water quality of the whole study. Composition of wastewater i.e. dilute wastewater shows high water consumption whereas concentrated wastewater shows low water consumption. Storm and rainwater further dilute wastewater which results in highly diluted wastewater (Henze & Comeau, 2008).

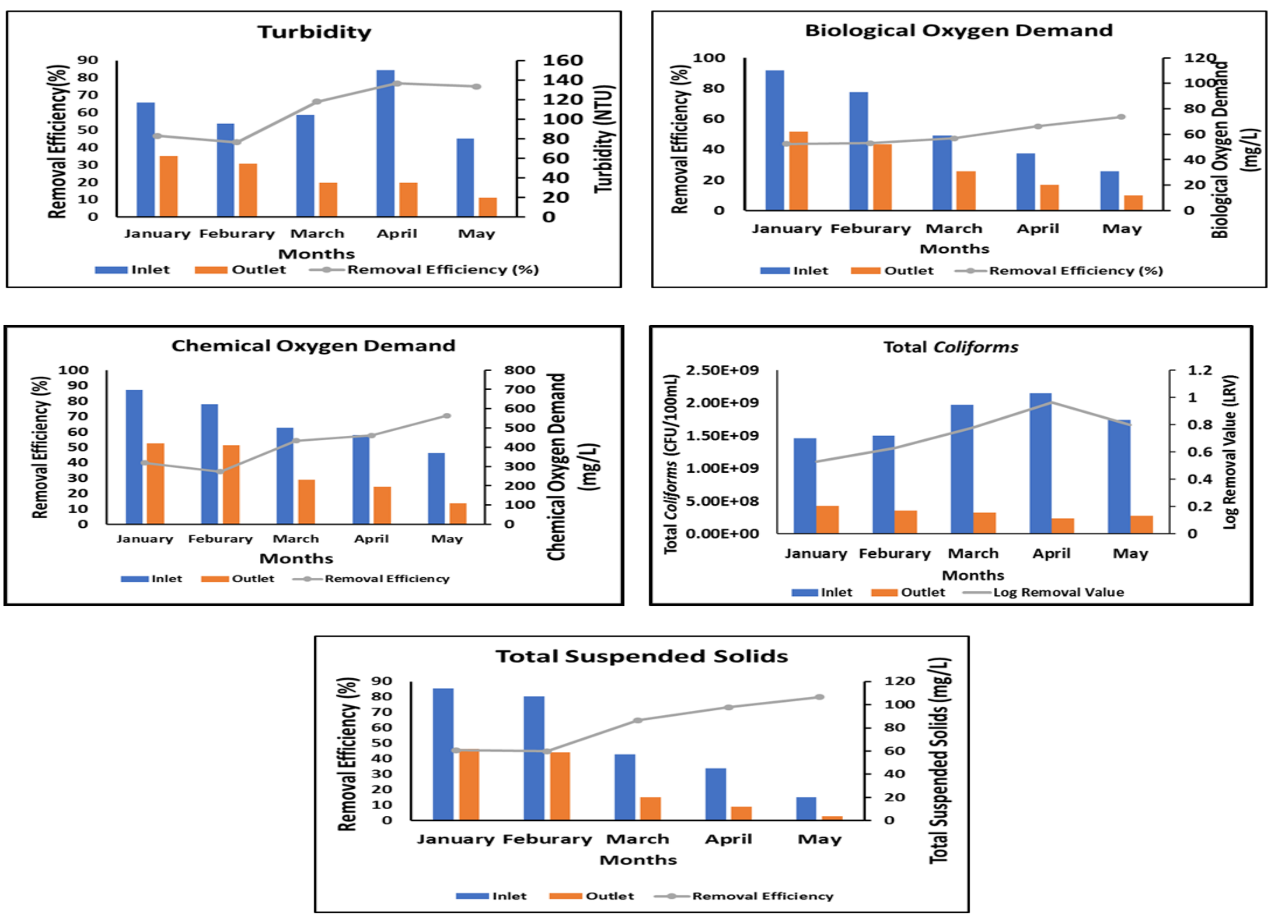

Figure 4.1 : Removal Efficiency (%) of Constructed Wetland

4.1.1 Turbidity:

Highest removal efficiency of turbidity was observed in April (76%), whereas lowest removal efficiency was observed in February (42%) as illustrated in Figure 4.1. Average removal efficiency of turbidity of constructed wetland was 46%, 42%, 66.3%, 76% and 75% for January, February, March, April and May respectively.

4.1.2 Biological Oxygen Demand:

Significant variation was observed in biological oxygen demand removal efficiency of constructed wetland. Removal efficiency is highly influenced by microbial degradation, biochemical activities of wetland. Minimum removal efficiency was observed in month of January with 43% whereas highest removal was observed in month of May with 61.2%. Removal of BOD is highly dependent on temperature; therefore, highest removal is observed during summer seasons as illustrated in Figure 4.1.

4.1.3 Chemical Oxygen Demand:

Removal efficiency of COD is highly influenced by microbial activity and organic load. Lowest removal efficiency (%) was observed in winters due to low temperature. Highest removal was observed in month of May with 70%. Whereas lowest removal (%) was observed in month of January and February with 39% and 34%. As plant density decreases during winter seasons which lead to low organic removal by constructed wetland as illustrated in Figure 4.1.

4.1.4 Total Coliforms:

Maximum *Coliforms* removal was observed in summer seasons, this is mainly due to macrophytes and high penetration of UV radiations. Weerakon and his coworkers reposted decline in Total *Coliforms* up to 6% due to drop in temperature. Maximum removal was observed in April with 89% whereas minimum removal was observed in January with 76% as illustrated in Figure 4.1.

4.1.5 Total Suspended Solids:

Maximum removal efficiency for Total Suspended Solids was observed in the month of May as 80%. This may be attributed to effective removal due to presence of well grown macrophytes. As temperature increases, rapid increase in removal efficiency was observed in month of March. In winters, minimum removal efficiency was observed in January (45%) and February (44%) due to high concentration of organic matter due to degradation of plants. as illustrated in Figure 4.1.

4.2 Evaluation of Physicochemical and biological parameters with Meteorological parameters:

Water quality parameters are being statistically analyzed in relation with climatic parameters. Meteorological data was acquired from US CASEN NUST including Ambient Temperature, Global Horizontal Irradiance, Relative Humidity (%), Wind Speed, and Air Absolute Pressure. Impact of these parameters has been discussed further in details.

4.2.1 Variation in water quality parameters with Global Horizontal Irradiance:

Solar radiations reach earth surface in several ways. Global Horizontal Irradiance is defined as sum of all short-wave radiation including Diffused Horizontal Irradiance and Direct Normal Irradiance. Where DIF is solar radiation that comes equally in all direction after scattering by particles and molecules and DNI is solar radiation that comes straight from sun to the sky (Geuymard, 2017).

A general trend of increase in Dissolved Oxygen is observed in low temperatures whereas it decreases in high temperatures. Monthly mean values of Dissolved Oxygen showed a strong negative correlation of Dissolved Oxygen of phytoremediation system and Global Horizontal Irradiance. During winter season, water has more ability to hold water contents as compared in warm seasons. Slight negative correlation of inlet (r= -0.51) and outlet (r= -0.89) of phytoremediation was observed as represented in figure 4.2-Annexure I. It is mainly due to less consumption of dissolved oxygen during low radiation.

Positive correlation was observed between GHI and pH of inlet (r= 0.27) of phytoremediation system. Whereas negative correlation was observed between GHI and pH of outlet (r=-0.73) of phytoremediation system as illustrated in figure 4.2. This is due to increase in GHI, improved efficiency of phytoremediation system resulting in more uptake of nutrients and reducing amount of organic matter which resulted in lesser pH of outlet of Phytoremediation system. Plant growth is highly retarded during less GHI which reduced efficiency of this biological system. Normal pH value ranges between 7-8 (Romanescu and Stoleriu, 2014). Seasonality in the pH of water is due to decay of debris as well imbalance of H+ ions. Decrease in pH is due to increase in vegetation and phytoplankton (Li *et al.,* 2015). pH between the range of 6-9 is considered well suited for the growth of macrophytes and microbes.

Increase in GHI resulted decrease in concentration of COD of phytoremediation system. A negative correlation was observed between Inlet (r= 0.99) and outlet (0.99) of phytoremediation system as illustrated in Figure 4.2. Reduction in COD is due to enhancement of self- diffusion efficiency which contributes toward its reduction. High COD is due to presence of high organic load (Vymazal, 2009). The present study showed high COD removal during summers as compared to winters due to increase in GHI.

Increase in GHI resulted in decline in concentration of BOD in phytoremediation system. A negative correlation was observed between inlet (r= 0.99) and outlet (r= 0.89) of wetland and GHI as illustrated in Figure 4.2. High BOD is mainly due to presence of high concentration of organic matter and debris. During rainfall season, decline in BOD is observed. It is mainly due to dilution of wastewater which decrease magnitude of BOD. As strong correlation is marked between rainfall and BOD (Mines *et al.,*2007).

Electric conductivity shows variation throughout the year. Electric conductivity range of wetland is crucial important factor because small variation in EC can change environment of whole phytoremediation system (Yu *et al.,* 2012). Electric Conductivity is due to presence of dissolved solids as a result electric current is produced in wetland.

Total Suspended Solids and Turbidity showed negative correlation with GHI as illustrated in Figure 4.2. As TSS mainly consists of clay, organic matter, inorganic matter and minerals which reduces transparency of water (Krithike, 2017). Whereas turbidity is directly related to reflection and absorption of light. High TSS was observed in winters due to high organic content as illustrated in Figure 4.2. Water transparency increases in summer season because of increase in GHI penetrating water surface (Abdel Satar, 2005).

Very strong positive correlation was observed between Total *Coliforms* in inlet (r=0.76) and GHI as illustrated in Figure 4.2. While a negative correlation was observed between Total *Coliforms* in outlet (r=-0.88) and GHI. Phytoremediation system is considered highly effective for removal of microorganisms.

4.2.2 Variation in water quality parameters with Relative Humidity:

Relative Humidity is defined as ratio of partial pressure of water vapors to the equilibrium vapor pressure of water at a given temperature. It is also dependent upon absolute pressure, air speed and ambient temperature of system. It is expresses as %, where higher percentage indicates high humidity level (Lawrence, 2005).

Significant fluctuation was noticed in Dissolved oxygen of both inlet and outlet of phytoremediation system. Positive correlation of r= 0.77 in inlet and r=0.74 in outlet with fluctuation in relative humidity as illustrated in Figure 4.3- Annexure I. Relative humidity is highest during winter season. This is in line with previous study by Sallam & Elsayed (2018). Maximum value of DO is observed during dry season due to temperature drop which lead to more winds resulting in solubility of oxygen in water. Similar results were reposted by Braid *et al., (2004)* in their study of water quality of water stream in Nigeria.

Fluctuation in relative humidity resulted in significant variation in pH of phytoremediation system. High pH is observed during high relative humidity whereas decline in pH was observed during low relative humidity. pH of inlet (r= 0.21) and outlet (r= 0.95) in correlation with Relative humidity as illustrated in Figure 4.3.

Biological Oxygen Demand is a measure of oxygen being used for biodegradation of organic matter by aerobic organisms. Significant positive correlation was observed between BOD of phytoremediation system and Relative Humidity as illustrated in Figure 4.3. BOD correlated with humidity, inlet r=0.86 and outlet= 0.73. High concentration of Biological Oxygen Demand shows that dissolved oxygen is depleted in system. High concentration of BOD lead to stress condition, resulting in death of organisms (Chindah *et al.*, 2011).

With the increase in relative humidity, significant increase in Chemical Oxygen Demand was observed as illustrated in Figure 4.3 As humidity varies throughout the year, similarly COD also varies. BOD correlated with humidity, inlet r=0.86 and outlet= 0.73. High value of COD is due to presence of excessive organic matter and concentration of organic matter is highly dependent upon microbial activities of degradation (Andleeb, 2017).

Electric Conductivity of this system is dependent upon humidity, evapotranspiration and temperature. (Caselles & Garcia, 2007). Non-significant correlation was observed between relative humidity and influent of phytoremediation system.EC is measure as ability of water to allow electric conduct to pass through it. This ability is dependent on presence of ions, their total concentration and temperature of water body. A correlation of inlet (r= -0.01) and outlet (r= 0.17) with relative Humidity.

Significant positive correlation was observed between TSS of inlet wastewater and outlet of phytoremediation system and relative humidity as illustrated in Figure 4.3. Similar results were discussed by Krithika and his coworker (2016) where Concentration of suspended solids were higher during high humidity due to low temperature. High concentration of TSS is due to high organic load leading to accumulation.

Significant positive correlation was observed between turbidity of inlet (r= 0.37) and outlet (r= 0.88) with relative humidity as illustrated in Figure 4.3. The water transparency value increase in summer as more solar radiations are allowed to penetrate surface water (Abdel Satar, 2005).Strong negative correlation was found between Total *Coliforms* and inlet (r= -0.91) of phytoremediation system. Whereas strong positive correlation was found between outlet (r= 0.62) of phytoremediation system and Relative Humidity.

4.2.3 Variation in water quality parameters with Ambient Temperature:

Temperature is one of important physical property that can directly influence rate of chemical reactions. Seasonal variation in temperature was recorded maximum during summer season and minimum during winter. Water temperature shows fluctuations due to geographical locations, climatic conditions, elevations and seasons. It also controls metabolism rate and activities of aquatic life (Murphy & Sprague, 2019).

Significant correlation is reported between microorganism's demand for oxygen and Temperature. Temperature was found to range between 13^0C and 25^0C. Maximum temperature was observed in the month of May and minimum in December. As temperature increases, concentration of Dissolved Oxygen reduces drastically (Virha *et al., 2011).* Similar results were observed in current study. A strong negative correlation was observed between inlet (r=0.46) and outlet (r=0.76) of Phytoremediation system and ambient temperature as illustrated in Figure 4.4- Annexure I. The temperature has direct influence on amount of dissolved oxygen, less oxygen is dissolved in warm water as compared to cold water.

pH is also a very important factor which as index of water pollution. As high and low pH is very harmful for aquatic life. Minimum pH was observed in January and it increased gradually with temperature variation. High temperature increases decomposition and decaying of plants as microbial activities increases during high temperature. Full plant growth plays a very important role in water treatment. Positive correlation was observed between inlet (r= 0.26) of phytoremediation system and Temperature whereas negative correlation of r= -0.75 was observed in outlet as illustrated in Figure 4.4. pH values keep fluctuating throughout but it important to keep pH of water in appropriate range from 6.5-9 (Bano *et al*., 2017).

Chemical Oxygen Demand is amount of oxygen being consumed by chemical breakdown of organic and inorganic matter. Increase in temperature enhance self-diffusion coefficient which contribute to reduction in Chemical Oxygen Demand. Many studies have reported negative correlation between COD and ambient temperature. In present study, a strong negative correlation was observed between COD and ambient temperature. As during dry seasons, water is more turbid which require high amount of oxygen for decomposition (Ranpise *et al.,* 2017). A correlation found in inlet and outlet of constructed wetland is r=0.98 and r=0.96 as illustrated in Figure 4.4. Higher COD was observed in January whereas lowest value was observed in May. High COD in water is mainly due to presence of concentrated organic matter due to sewage discharge, which is dependent on microbial activity of anaerobic and aerobic bacteria (Vymazal, 2009).

Biological Oxygen Demand is also a very important parameter which indicates magnitude of Oxidizable organic pollutant. A strong negative correlation was observed between inlet (r=-0.99) and outlet (r=-0.89) of phytoremediation system and temperature as illustrated in Figure 4.4. BOD of inlet sample is comparatively higher to outlet sample. A higher BOD shows high consumption of oxygen and high concentration of organic pollutant. Maximum reduction in BOD is observed during summer season as temperature increases microbial activity. COD and BOD both are significantly correlated but COD values are always higher than BOD (Klimas *et al., 2016).*

Electric Conductivity is ability to carry electric current. EC of phytoremediation system keep fluctuation during whole period. EC of outlet sample was >3500 mg/L, a permissible limit set by EPA to fit for agricultural purpose. Non-Significant correlation was observed between outlet of phytoremediation system and Temperature as illustrated in Figure 4.4. Similar results were quoted by Andleeb (2017). Warm water has more viscosity which allows electric current to move freely.

Significant negative correlation was observed between turbidity of outlet (r=-0.97) and ambient temperature whereas nonsignificant correlation was observed between turbidity of inlet and temperature as illustrated in Figure 4.4. Turbidity is highly influenced by presence of suspended particles, light reflection and morphology of area. (Mustapha *et al., 2013).* Water transparency showed a negative correlation with Total Solids Biological Oxygen Demand and Chemical Oxygen Demand (Abdel Satar, 2005).

Whereas TSS also shows a negative relation with Ambient temperature. Maximum value of TSS was observed during January and minimum in May. Negative correlation was observed between inlet (r=1) and outlet (r=0.9) with temperature as illustrated in Figure 4.4. There is reduction in TSS in outlet sample due to microbial activity and filtration (Ramakrishna *et al.*, 2013). High TSS is reported in winter seasons due to presence of debris, floating particles (Krithika *et al*., 2016).

Positive correlation was noticed between total *Coliforms* concentration in inlet (r= 0.60) and ambient temperature as illustrated in Figure 4.4. Total *Coliforms* concentration increases with increase in temperature. As temperature increases above optimum level, concentration of total *Coliforms* starts decreasing. Highest concentration of TC was observed in April. Whereas, negative correlation was observed between TC of outlet and ambient temperature (Andleeb, 2017).

4.2.4 Monthly Shift in water quality parameters with Wind Speed:

Wind mixing in water body is very important because wind induce sediments resuspension which directly influence water quality. A strong wind has a great power in water body as it can help in mixing and in light penetration in water body (Jin *et al.,* 2018).

Gas transfer velocity is highly influenced by wind speed which in result increases concentration of gases in water. Similarly, a positive correlation was observed between Dissolved oxygen of inlet and Wind speed. As wind speed increases, concentration of dissolved oxygen in water also increases. Following results are also supported by Cao and his coworkers, where it is found that weather also influence Dissolved Oxygen. A strong correlation (r= 0.66) was found between windspeed and dissolved oxygen.

A strong positive correlation was observed between Turbidity of inlet sample and Wind Speed as illustrated in Figure 4.5. Similar strong positive relationship was found in a research conducted by Yang and his worker (r= 0.75) between turbidity and wind speed. Variation in Turbidity is mainly due to the presence of particle composition (organic, inorganic, fine particles, and microorganisms) throughout the year (Yang *et al.,* 2019).

Strong positive correlation was observed between Total *Coliforms* and Wind Speed as illustrated in Figure 4.5. As stronger wind easily generate force in water body which induce Total *Coliforms* growth. Wind induced water mixing has strong impact on microbial growth (Lu *et al.,* 2019). Whereas strong negative correlation was observed between Total *Coliforms* of inlet and windspeed, resulting in efficient microbial removal efficiency.

Mass concentration of solids increase with increase in wind speed in Total Suspended Solids. it is mainly due to sediments resuspension resulting in increase in total suspended solids (Behrooz *et al.,* 2016). Positive correlation was observed between TSS of outlet of phytoremediation system and wind speed. Despite that, similar results were not observed in April and decline in total suspended solids was observed. Whereas, negative correlation was observed between TSS of outlet and Wind speed.

A strong correlation was observed between Biological Oxygen Demand of phytoremediation system and Wind Speed. Results showed a positive correlation between inlet (r= 0.76) and outlet (0.87) of constructed wetland and wind speed. Whereas, a strong negative correlation was observed between Chemical Oxygen Demand of wetland and windspeed where r= -0.74 for inlet and r=-0.58 for outlet as illustrated in Figure 4.5.

pH of inlet sample showed a linear relation with wind speed as illustrated in Figure 4.5. As Wind speed increased, increase in pH was observed. This may be attributed to sediments resuspension resulting increase in pH of wastewater. Whereas negative relation was observed between pH of outlet sample and wind speed.

Electric Conductivity is ability of water to allow current to pass through it. Electric Conductivity of phytoremediation system is depended on evapotranspiration and temperature (Caselles-Osorio and Garcia, 2007). A very weak correlation (r=-0.09) was observed between EC of inlet sample and windspeed as illustrated in Figure 4.5. This may be attributed as non-significant relation.

4.2.5 Variation in water quality parameters with Absolute Pressure:

Solubility of any gas in liquid is highly influenced by pressure of that gas about solution surface. Many gases like oxygen dissolved easily in water under high pressure than low pressure. Therefore, more oxygen dissolved at sea level (Sander, 2015).

Due to direct relation between absolute pressure and oxygen solubility, a strong positive correlation was observed between inlet (r= 0.55) and outlet (r= 0.55) of phytoremediation system and absolute pressure. Electric Conductivity of inlet sample showed a weak negative correlation with pressure. Whereas positive correlation (r= 0.12) was observed between EC of outlet sample and pressure. This may be attributed to increase in EC due to temperature increase.

A strong positive correlation was observed in biological oxygen demand of inlet (r=0.9) and outlet (r= 0.85) of phytoremediation system with absolute pressure as illustrated in Figure 4.6. Similar results were observed in correlation of COD of constructed wetland with absolute pressure. A strong positive correlation was observed in chemical oxygen demand of inlet (r=0.93) and outlet (r= 0.71) of phytoremediation system with absolute pressure.

Insignificant negative correlation was observed between pH of inlet sample and absolute pressure. Whereas pH of outlet sample showed a strong positive correlation with absolute pressure.

A strong positive correlation was observed between turbidity of phytoremediation system and absolute pressure as illustrated. Whereas an exception was observed in month of April. Correlation of r=0.22 was observed between turbidity inlet sample and pressure where as positive correlation of r= 0.85 was observed between turbidity of outlet sample and pressure. Similar positive correlation was observed between TSS of phytoremediation system and pressure as illustrated in Figure 4.6. Positive correlation of r=0.84 was observed between TSS of inlet sample and pressure where as positive correlation of r= 0.77 was observed between TSS of outlet sample and pressure.

Strong negative correlation was observed between Total *Coliforms* and Wind Speed as illustrated in Figure 4.6. Correlation of r=0.84 was observed between Total *Coliforms* of inlet sample and pressure where as positive correlation of r= -0.8 was observed between Total *Coliforms* of outlet sample and pressure.

4.3 To determine the Helminth parasites in treated and raw wastewater in Constructed Wetland

Water samples were weekly collected from inlet and outlet points of constructed wetland for microscopic analysis for period of 5 months starting from January 2018 to May 2018.

4.3.1 Identification of Helminth Parasites Eggs

Identification and enumeration of helminth eggs in water is very necessary for infection level in environment. As presence of high helminthic parasites is mainly linked with inadequate sanitation and health facilities and also poverty. (Mahvi *et al.*, 2006). Microscopic observation of effluent water indicated a variety of helminths parasites in inlet water samples and treated water. Most predominant helminths species found were *Ascaris lumbricoides*, *Hook worms*, *Trichuris trichuria*, *Hymenolepis nana* and *Enterobius vermicularis*. Recommended limit for helminth eggs is <1 egg/Liter. These eggs are mainly common in wastewater in urban environment (Grego *et al.*, 2018). Parasitic eggs count was relatively lower in treated water. Following pie chart below shows percentage presence of helminths eggs in wastewater of constructed wetland.

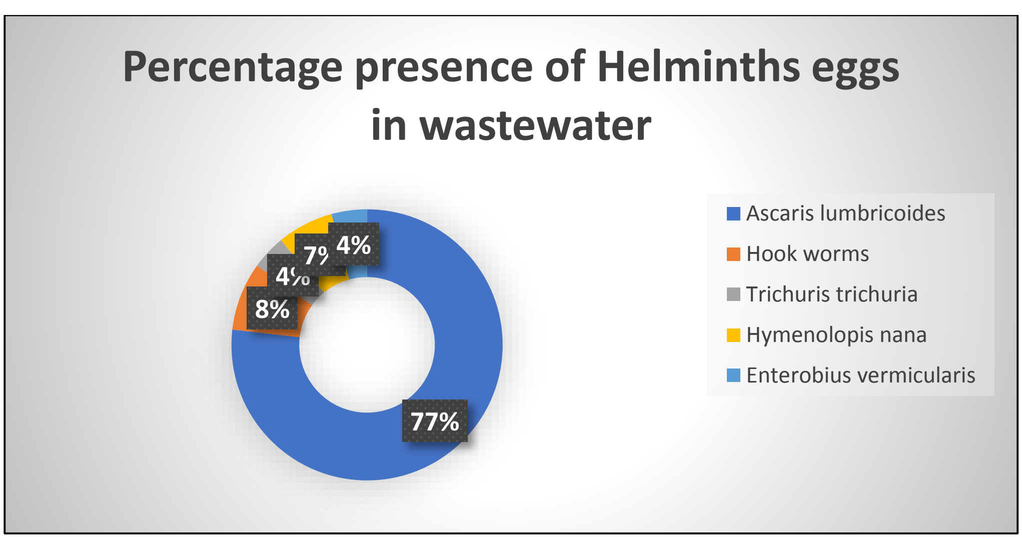

Figure 4.7: Percentage Presence of Helminths eggs

Type diversity of helminth eggs is very important to look at their seasonal distribution and impact of environmental factors i.e. temperature, rainfall and sunshine. High diversity is mainly observed during favorable environment i.e. wet season with maximum humidity and temperature for eggs development (Abagale *et al.*, 2013).

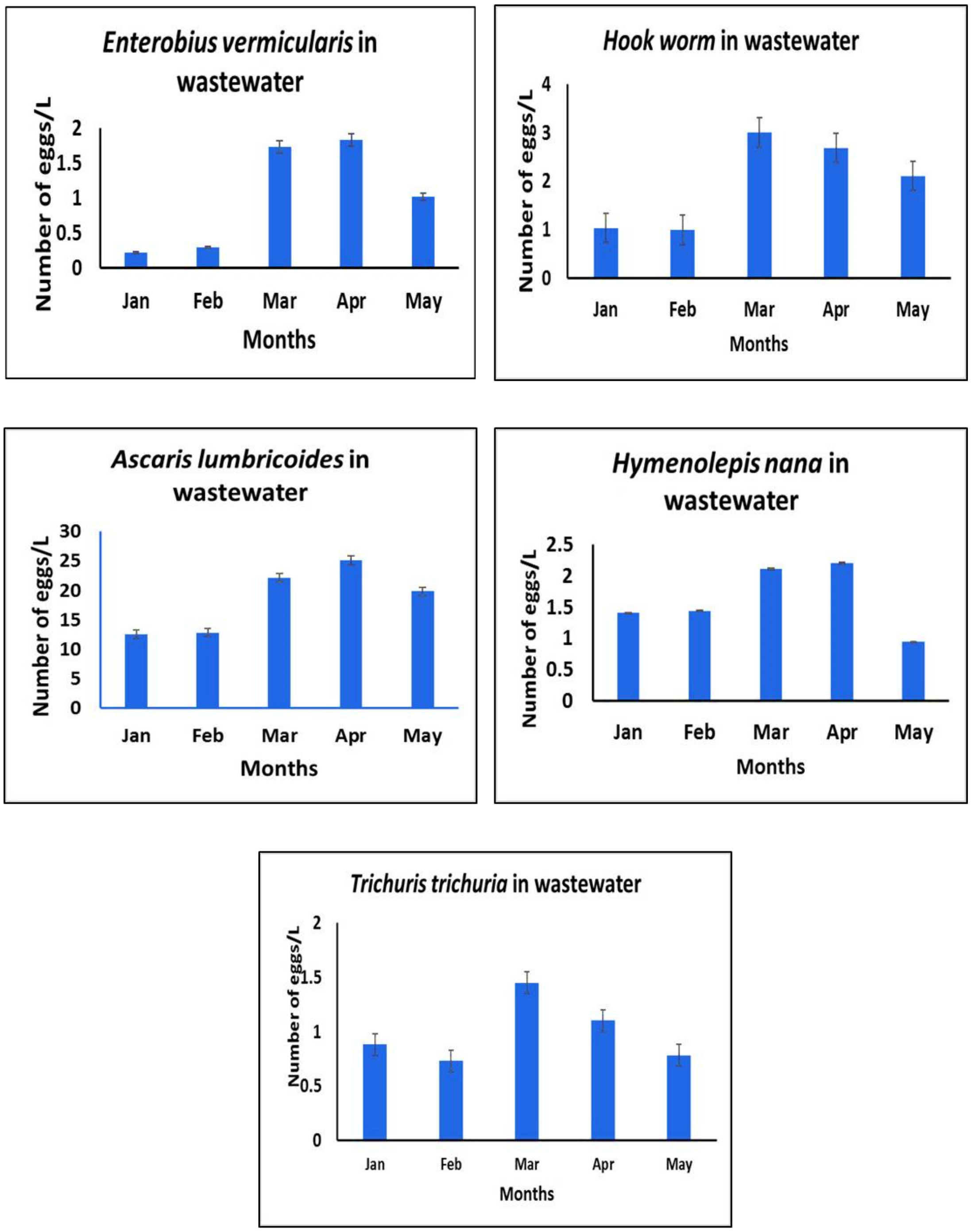

Figure 4.8 : *Major Helminth parasites* in wastewater

4.3.1.1 *Ascaris lumbricoides*:

Most abundant and persistent specie found in water sample. Up to 77% of *Ascaris lumbricoides* were observed in total wastewater sample. High concentration of *Ascaris* lumbricoides is mainly attributed to high resistance toward external conditions which allow it to remain viable for longer. It is among commonest parasites in developing countries, found to be abundant in domestic wastewater (Yaya-Beas *et al.,* 2016). Number of eggs per liter showed variation throughout sampling time. As temperature increases, significant increase in concentration was observed followed by decline in the month of May. It may be due to inability to survive above optimum temperature. Minimum number of eggs were observed in January (12.5/L) followed by February (12.85/L) as illustrated in Figure 4.8. High concentration of eggs was observed in April (25.1/L) which may be attributed to rainfall. Similar results were reported by another study with presence of *Ascaris lumbricoides* as most abundant specie with 88.3 % (Chaoua *et al.,* 2018).

4.3.1.2 *Hook worm*:

Followed by *Ascaris lumbricoides, Hook worm* eggs were found highest among all. It is considered different from highly potential pathogenic eggs. Due to its large size, it mainly adheres to surface which help to settle easily. (Yaya- Beas *et al.,* 2016). Fluctuation was observed in eggs per liter throughout sampling. Up to 8% of eggs were observed in total wastewater sample Maximum eggs were counted in month April (2.63/L) due to rainfall. Whereas lowest count of eggs was found in January (1.03/L) followed by February (0.99/L). A significant increase in number of eggs was noticed in March (3/L), in which optimum temperature was observed.

4.3.1.3 *Hymenolepis nana*:

Up to 7% of eggs were observed in total wastewater sample. Similar trend was observed in eggs per liter. Highest concentration of eggs was observed in the month of March (3/L) due to favorable environment. January and February show minimum count of 1.4/L and 1.43/L *Hymenolepis nana* eggs. Motevalli and his worker also found *Hymenolepis* nana among abundant nematodes during performance evaluation of artificial wetland (Motevalli *et al.,* 2015).

4.3.1.4 *Trichuris trichuria:*

It is also an important helminthic parasite mainly observed in wastewater samples. Up to 4% of *Trichuris trichuria* were observed in total wastewater sample helminths eggs. Similar results were observed in study conducted by Chaoua and his worker where parasitological analysis of wastewater showed presence of *Trichuris trichuria as* 3.53% (Chaoua *et al.,* 2018). Lowest count of 0.21/L and 0.29/L was observed in the month of January and February. Whereas, highest count of 1.45/L was observed in month of march followed by 1.1/L in April.

4.3.1.5 Enterobius vermicularis:

It is another disease-causing helminth parasite which was seen in wastewater effluent. It is among most predominant species of helminths eggs in wastewater (Yaya-Beas *et al.*, 2016). Maximum count of eggs was observed in month of March (1.73/L) and April (1.83/L) due to favorable environment. Whereas minimum number of eggs were found in January (0.2/L) and February (0.29/L). up to 4% of *Enterobius vermicularis* was observed in total helminth eggs count. Prevalence of *Enterobius vermicularis* agrees with a previous study in Nigeria where of *Enterobius vermicularis* was found among most common parasites in vegetables sample (Chijioke *et al.*, 2018).

4.3.2. Quantitative characterization of Helminths eggs in Constructed wetland:

Presence of parasitic helminths eggs in wastewater with disease causing pathogens is a major health concern which is often neglected. These eggs are mostly excreted through feces and it spread through water, food and soil. They are highly persistent to environment. A large concentration of helminths eggs was observed in wastewater. Following table below shows concentration of helminths eggs in wastewater.

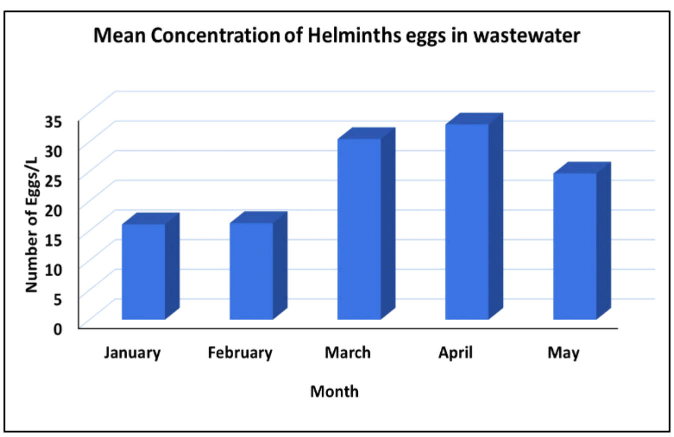

Figure 4.9(a): Mean Concentration of Helminths eggs in wastewater

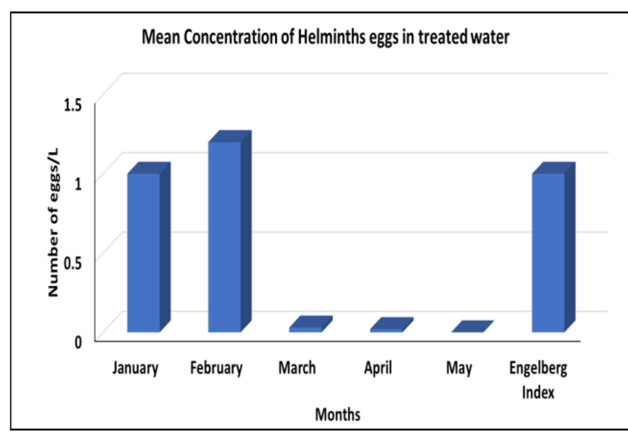

Figure 4.9(b): Mean Concentration of Helminths eggs in treated wastewater

Raw water samples contained different types of helminths parasites. According to World Health Organization, Concentration of helminth eggs is one of important water parameters, as an indicator of sanitation risk related to reuse of treated water for irrigation purpose. According to WHO, concentration of helminths eggs should be less than or equal to 1 for safe use of water for irrigation purpose. Result shows high concentration of helminth eggs in inlet wastewater. Significant variation was observed in eggs within 5 months from January to April. Mean concentration of helminth eggs in January was 16 eggs/L and 16.26 eggs/L in February. However, the differences were not significant. Whereas a significant increase was noted in March with 30.44 eggs/L followed by 32.9 eggs/Liter in April. This increase in helminth eggs is attributed to increase in temperature which resulted in increase in number of microorganisms (Molleda *et* *al.*, 2008). It further declined to 24.6 eggs/L in May. The presence of Helminth eggs is also dependent upon size of community served and disease rate within community (Sabbhai *et al.*, 2018). As major source of water is academia and residential, helminth eggs detected in study were likely of human origin. Similar result in wastewater are reported by Ajonina *et al.* (2015). High number of helminths shows a disease risk factor to public health. These helminth eggs are highly persistent in environment, which make them highly resistent to ozone, chlorine and UV light.

Result showed maximum removal of helminths eggs in treated water. Variation is mean concentration was observed throughout sampling. Removal of efficiency of constructed wetland improved with increase in temperature. In March, followed by April, number of helminth eggs in wetland was >1. This shows effectiveness of constructed wetland and use of treated water for agriculture purpose. Similar results are reported by Motevalli *et al.* (2015) where number of nematodes eggs were found less than 1 per Liter. Reason of reduction in helminths eggs is mainly due to effective settling of helminths eggs due to sedimentation. Also, solar radiation, temperature and presence of predator species. Another study by Paruch found that Rhizomes of commonly used macrophytes also enhance removal by helminths eggs by 100%. Extensive root structure helps in purification of water. Constructed Wetlands plants help in parasite removal by providing large surface area for attachment of microbes to the roots structure (Shingare *et al.*, 2017).

4.2.2 Removal efficiency of Constructed wetland for Helminths parasites:

Up to 100% removal of helminths eggs was observed in the wetland, which shows removal efficiency of wetland. Similar results were found in study conducted on artificial wetland, where study showed 100% removal of nematodes eggs (Motevalli *et al.*, 2015).

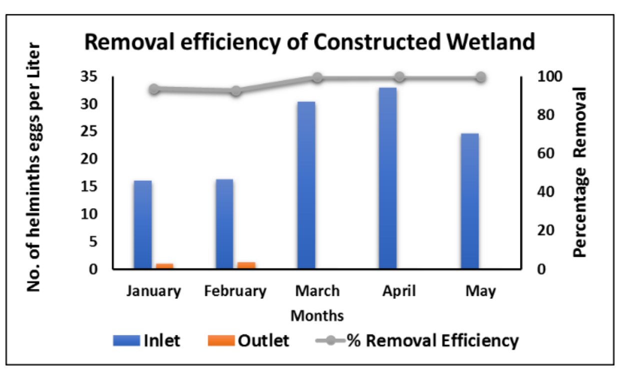

Figure 4.10: Removal efficiency of constructed wetland for Helminth parasites

Molleda *et al.* (2008) also conducted a study on removal of pathogenic wastewater indicators by constructed wetland. Low helminths egg count was reported with up to 100% removal during warm season. Similar results are observed in current study, increase in removal efficiency was observed with increase in temperature.

4.4 Evaluate concentrations of metals in Constructed Wetland to provide an insight into the current pollution status

4.3.1 Metal concentration wastewater and removal efficiency:

Table 4.2: Removal Efficiency (%) of Constructed Wetland

Trace Metals	Inlet	Outlet	Removal Efficiency (%)
Zinc	0.21	0.1	52.4
Lead	0.1	0.02	80
Manganese	0.1	0.03	70
Iron	0.71	0.2	71.8

Zinc:

Concentrated wetlands are highly preferred due to its Zn removal from wastewater. Very minimal change was observed in metal concentration. 0.21 mg/L concentration of Zn was observed in wastewater of constructed wetland. Whereas 0.1 mg/L was observed in outlet water. Study showed removal efficiency of 52.38%. Kröpfelová *et al.*, also obtain 58.3% removal efficiency for Zn removal. Concentration of Zinc in wastewater was within permissible limit for irrigation purpose. Recommended value of Zinc for irrigation water is 5mg/L (NEQS, 2000).

Iron:

Iron concentration in wastewater was found within permissible limit of NEQS. Recommended level of Fe is 2 mg/L. whereas 0.7mg/L of iron was observed in incoming wastewater whereas 0.21mg/L was observed in outlet water. Up to 71.8% removal efficiency was observed. Iron concentration also lied below WHO (1.0mg/L).

Lead:

0.1 mg/L of lead was found in wastewater of wetland. Concentration of lead in wastewater was observed higher than WHO permissible level (0.05). whereas 0.02 mg/L of lead was found in outlet water. Lead concentration was found within permissible limit of National Environmental Quality Standards (0.5mg/L). The removal efficiency of Constructed wetland was 80%, higher than 50% reported by literature (Khan *et al.*, 2009).

Manganese:

Very small concentration up to 0.1 mg/L of Mn was found in wastewater. Whereas 0.03% of manganese was found in outlet water. Up to 70% removal efficiency was observed in Manganese in constructed wetland. Plants have ability to take heavy metals which lead to effective removal (Tjandraatmadja & Diaper 2006).

4.3.2 Metal Accumulation in Plant Tissue:

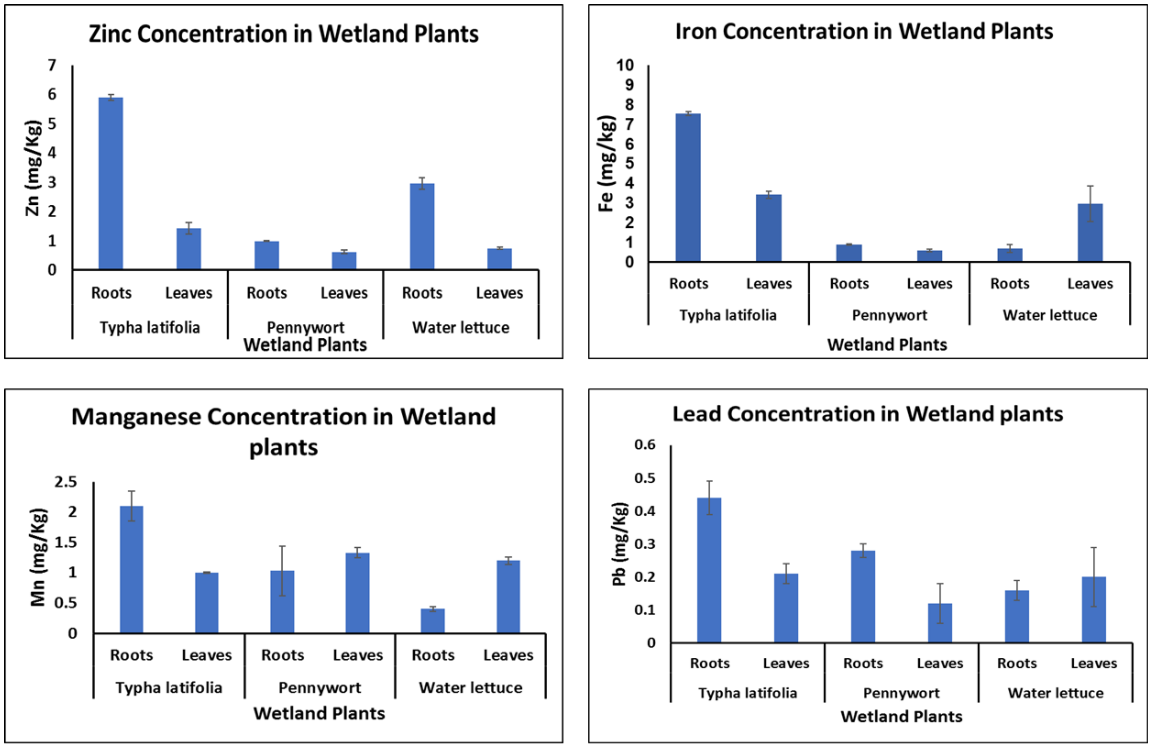

Figure 4.11: Metal concentration in Constructed Wetland plants

4.3.2.1 Iron:

Result showed high uptake of Fe by *Typha latifolia*. Overall high concentration of iron was found in all plants. Highest concentration of iron was observed in month of July (7.53 mg/kg). Presence of long roots and thick root hairs make *Typha* highly effective for removal of metals from wastewater (Sricoth *et al.*, 2018). Very high concentrations of iron were observed in roots as compared to leaves, this may indicate that roots acts as a physical barrier which limits transfer of Fe to above ground leaves (Angnassa *et al*., 2019). High uptake of Iron was observed during July, this may be due to growing season of plants. Roots play a significant role in uptake of metals. Where as in case of water lettuce, high concentration of Iron was found in leaves, this may be attributed to involvement of roots in transportation whereas leaves are primarily involved in accumulation of metals. High growth rate of water lettuce also makes it an efficient plant for removal of metals from wastewater (Rezania *et al.,* 2015).

4.3.2.2 Zinc

Zinc is considered an important micronutrient for metabolism and plant growth. Concentration of zinc in plants was also within permissible limit. Whereas high concentration of Zinc was found in roots as compared with leaves. *Typha* shows 5.9 mg/Kg Zinc in roots whereas in case of pennywort, 0.99 mg/Kg was observed. Similar trend was noted in water lettuce with maximum concentration of Zn (2.96 mg/Kg) in roots whereas lower concentration was observed in leaves (0.74). This also corroborates previous research findings that shows high tolerance of *Typha* to metal uptake (Bonannno and Cirelli, 2017). Macrophytes have capacity to uptake essential micronutrients than toxic elements. High concentration of these micronutrient may become toxic if exceed limit. Elemental compartmentation of plants acts as a tolerance strategy to prevent its translocation to photosynthesis parts. Small variation was observed in elemental uptake of plants due to seasonal changes. Water lettuce and pennywort are considered as cheap and best cleanup technology for contamination of soil and wastewater (Vongdala *et al.*, 2019).

4.3.2.3 Lead:

Lead is not considered as an essential metal for plant growth and it is considered highly toxic. Highest tolerance to lead was found in *Typha (*0.44 mg/Kg). High tolerance of Pb in lead has been reported by Bonanno and Cirelli. High accumulation of lead in roots prevent translocation in to leaves due to its immobilization in soil. Moreover, it is also reported that lead concentration mainly found in leaves is due to wind contamination rather than soil (Bonanno *et al.,* 2018). Iron plaque also act as a barrier which prevents uptake of toxic metals in plants body. Similar results were found in pennywort with highest uptake of Pb (0.28 mg/Kg) in roots and 0.12 mg/kg in leaves.

4.3.2.4 Manganese:

It is also an important micronutrient for plants growth and metabolism. High concentration of it may become toxic to plants. Manganese content in *Typha* was higher as compared to other plants. Highest concentration of Mn was observed in roots of *Typha* with 2.1 mg/Kg whereas lowest concentration of Mn was observed in roots of water lettuce. This may be due to involvement of roots in transportation whereas leaves are primarily involved in accumulation of metals. Mn is a typical element which occurs in urban wastewater as a disinfectant. High accumulation of trace elements in roots suggest presence of chemical species in sediments which blocks toxic metals from translocation to plant’s aboveground organs.

5. Conclusion and Recommendations

5.1 Conclusion

1. Removal efficiency of constructed wetland was 80%, 89%, 70%, 61.2% and 75% for Total Suspended Solids, Total *Coliforms*, Chemical Oxygen Demand, Biological Oxygen Demand, and turbidity.
2. A strong relationship was observed between selective meteorological parameters (Global Horizontal Irradiance, Ambient Temperature, Absolute Pressure, Wind Speed and Relative Humidity) and water quality parameters (TSS, BOD, COD, DO, pH, Turbidity and Total *Coliforms*.
3. High concentration of Helminth parasite eggs was found in inlet wastewater. Most predominant helminths species found were *Ascaris lumbricoides*, *Hook worms*, *Trichuris trichuria*, *Hymenolepis nana* and *Enterobius vermicularis.* Up to 100% removal of helminths eggs was observed in the wetland, which shows removal efficiency of wetland.
4. Highest uptake of trace metals was observed in *Typha latifolia* due to its high biomass and efficient root system. Highest concentration of Iron was uptake from all wetland plants during July. Highest concentration of metals was observed in roots than leaves and shoots.

5.2 Recommendations

1. Harvested plants should be subsequently assayed for energy efficiency, i.e., calorific value to determine wetland plant waste may use as serve as an alternative energy source in developing countries like Pakistan
2. Further research should be conducted on accumulation pattern of contaminants in different compartments of wetland plants
3. Dissolved Solids influence on the heavy metal behavior on different substrate materials and on plants (e.g., physiological characteristics) used in CWs should be further studied
4. Detailed study on production of biofuel by direct combustion of dry biomass need to be done

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Annexure

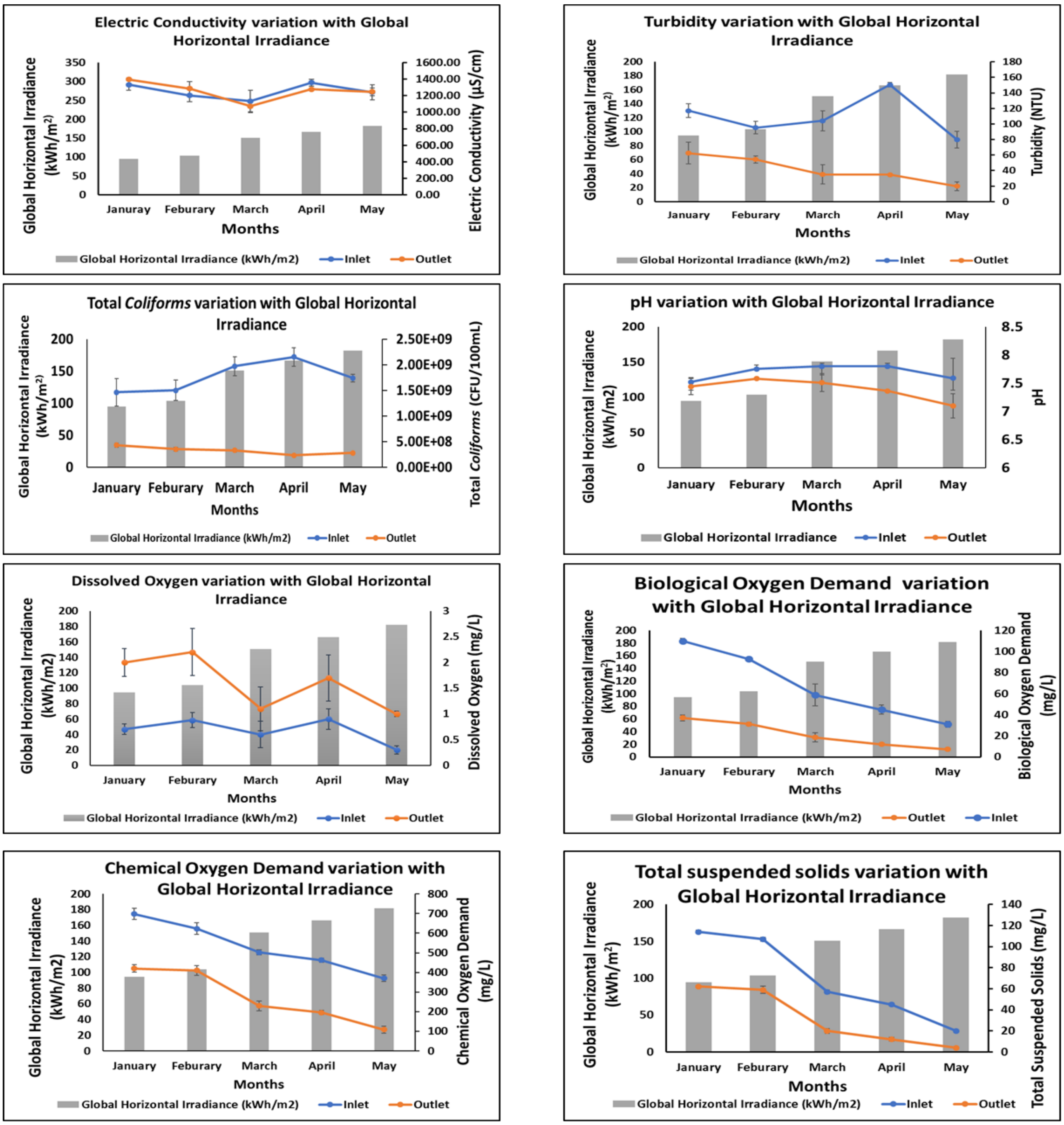

Figure 4.1: *Variations in Water Quality Parameters with Global Horizontal Irradiance*

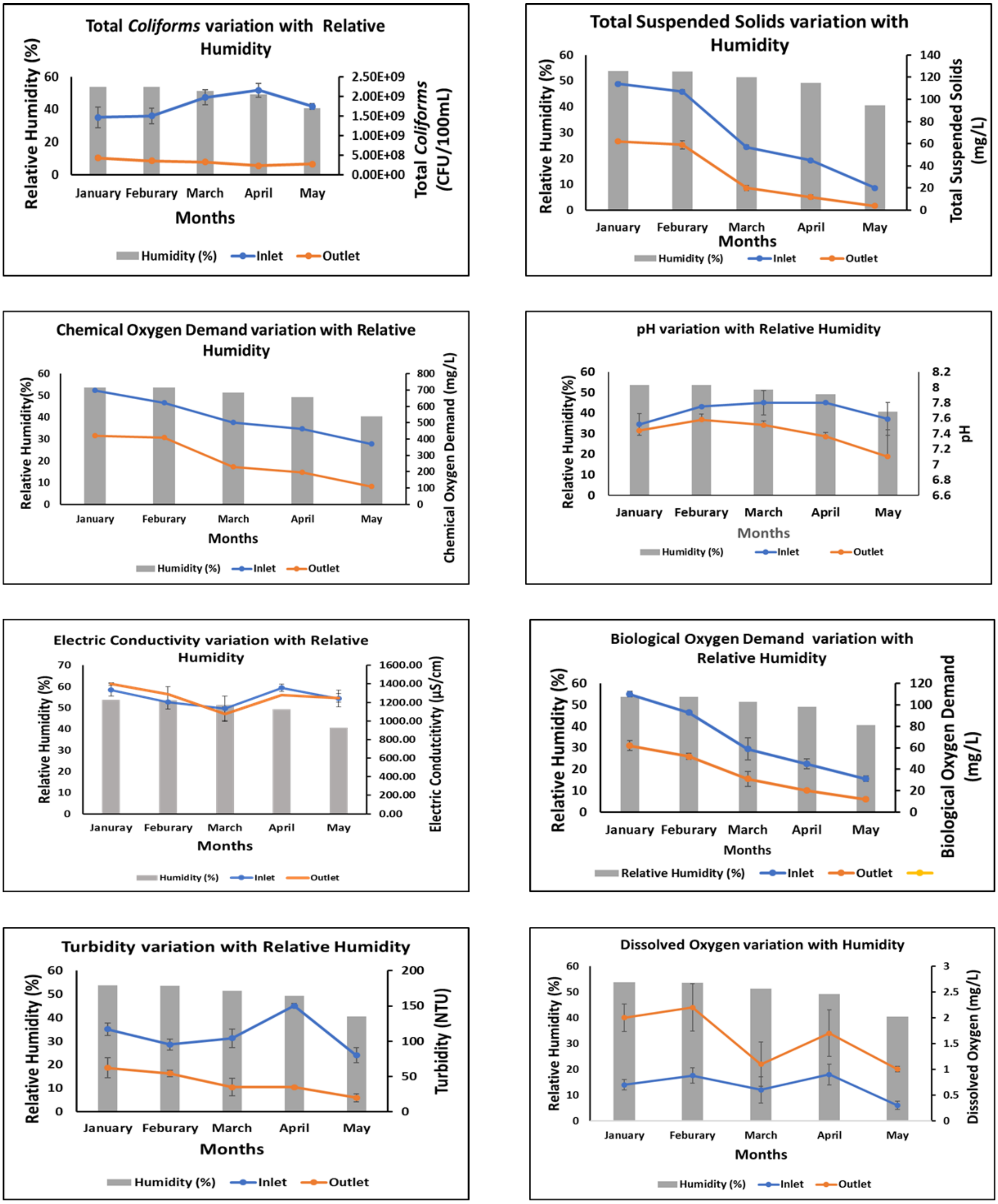

Figure 4.2: *Variations in Water Quality Parameters with Relative Humidity*

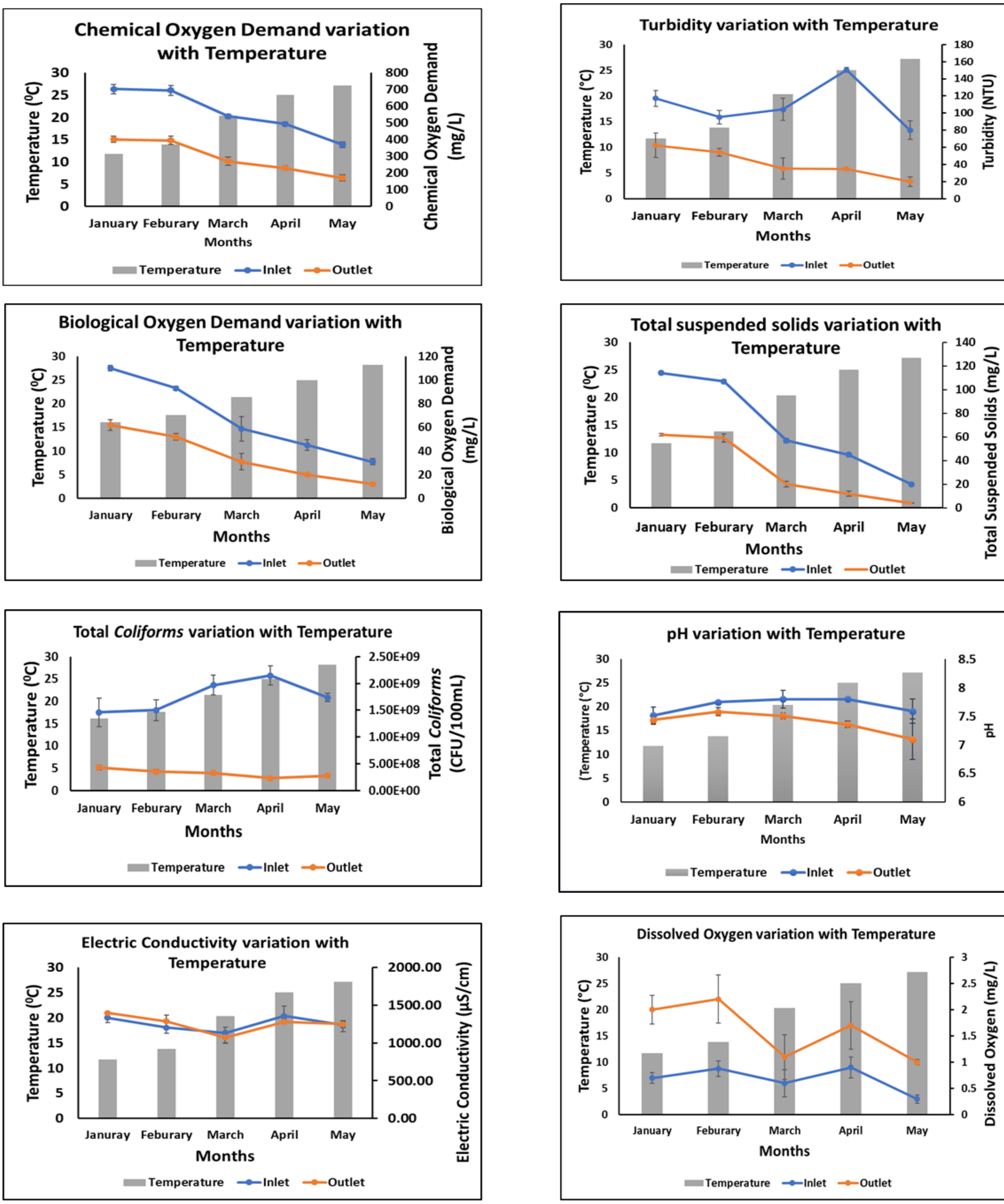

Figure 4.3: *Variations in Water Quality Parameters with Ambient Temperature*

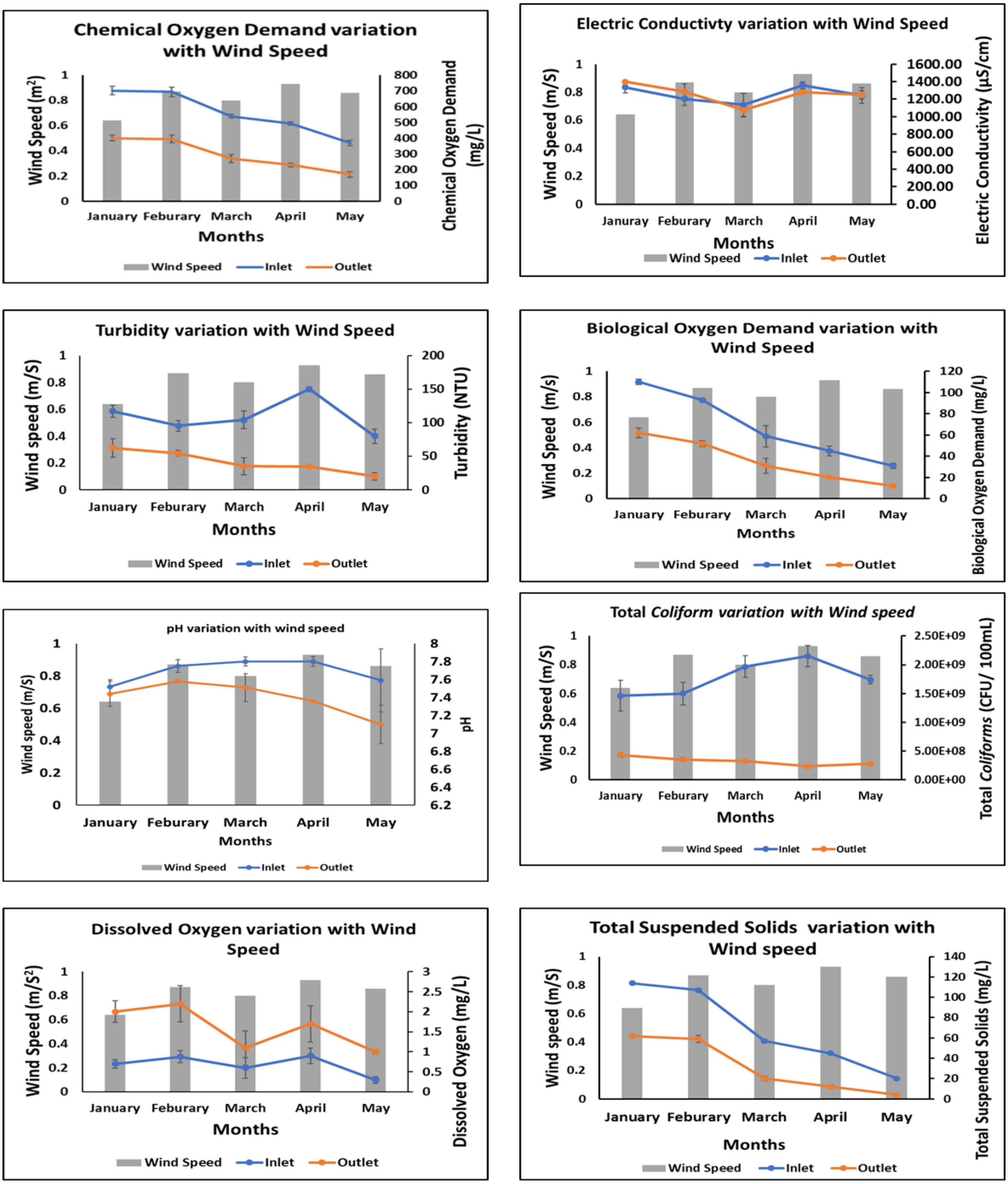

Figure 4.4: *Variations in Water Quality Parameters with Wind Speed*

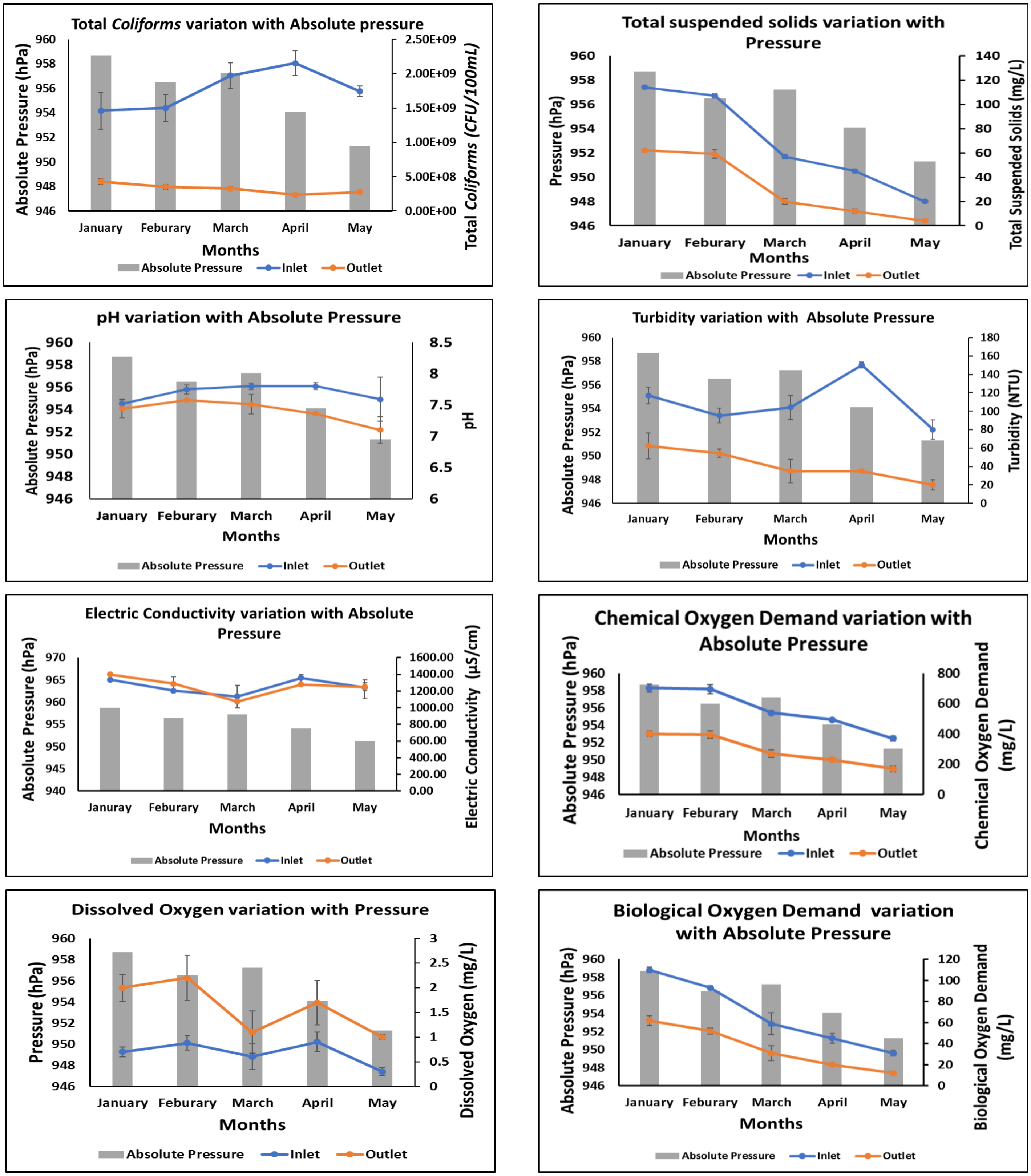

Figure 4.5: *Variations in Water Quality Parameters with Pressure*