Removal of Pollutants from Integrated Constructed Wetland

Submitted by

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This Dissertation is Dedicated to My Ever-Loving Mother,

Encouraging Father & Husband, caring Brothers & Sisters

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List of Abbreviations

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As	Arsenic
Cd	Cadmium
COD	Chemical Oxygen demand
Cr	Chromium
DNI	Direct Normal Irradiance
DO	Dissolved Oxygen
EC	Electrical Conductivity
FBT	Food-borne illness T*rematodiasis*
FILTER	Filter and Irrigated Cropping for Land Treatment and Effluent Reuse
GBD	Global Burden of Diseases
HEAD	Helminths Eggs Automatic Detector
Hg	Mercury
HSSF-CW	Horizontal subsurface flow – Constructed Wetland
ICWs	Integrated Constructed Wetland System
NH^{4+}-N	Ammonium-Nitrogen
NO^{3-}-N	Nitrate-Nitrogen
NO^{2-}-N	Nitrite-Nitrogen
P1	Pond 1
P2	Pond 2
P3	Pond 3
P4	Pond 4
P5	Pond 5
P6	Pond 6
P7	Pond 7
P8	Pond 8
Pb	Lead
PO_4^{3-}-P	Phosphate-Phosphorus
rpm	Revolution per minute
SDGs	Sustainable Development Goals
TDS	Total Dissolved solids
TKN	Total Kjeldahl Nitrogen
TSS	Total suspended solids
VF-CW	Vertical flow – Constructed Wetland
WHO	World Health Organization

Abstract

Water is contaminated when it is affected by the ejection of anthropogenic pollutants. A simple, cost effective and environment friendly integrated constructed wetland system (ICWs) at NUST Islamabad was studied from September 2019 to February 2020 to evaluate the treatment efficiency of domestic wastewater implanted with following plant species: *Typha latifolia*, *Centella asiatica*, *Pistia stratiotes*. The present study was conducted to produce effluents with minimum organic contents that render it harmless to the receiving agricultural land or water bodies with special reference to the parameters of pH, Temperature, Dissolved Oxygen (DO), Turbidity, Nitrates, Chemical Oxygen Demand (COD), Phosphate, Total Dissolved Solids (TDS) and Total Suspended Solids (TSS). Furthermore, this study explains the removal efficiency of enteric helminths eggs and heavy metals presence in wastewater, sediments, and plants. The results revealed that the maximum removal efficiency of the above-mentioned parameters of COD, Turbidity, Nitrate, Phosphates, TDS, TSS was 97% (November), 84% (December), 99% (October), 95% (October), 38% (October), 91% (November) respectively. Thus, average removal efficiency was higher in autumn (October-November) as compared to winter (December-January). The possible reason for this may be explained to be as the condition of moderate temperature required for vigorous action of microorganisms. This may also be attributed to the need of well grown plants to actively grow in the ICWs at moderate temperature conditions during these months. Helminths Eggs Automatic Detector (HEAD) automatic system was used to effectively quantify and identify helminths eggs commonly with steady results i.e., *fertile and infertile eggs of Ascaris spp. (lumbricoides and suum)*, *Trichuris trichiura*, *Toxocaracanis, Hymenolepisdiminuta, Hymenolepis nana, Fasciola hepatica, Schistosoma mansoni*, and *Taenia saginata* in wastewater, sediments, and plants samples. However, 100 % average removal efficiency of helminths eggs was observed from September to December and 50 % in January and February. Successful pathogens removal has been observed using ICWs and hence considered as the suitable wastewater treatment systems. It was concluded that the ICWs performed well and has a considerable potential of the removal of pollutants from wastewater.

Keywords: Integrated Constructed Wetland, Domestic Wastewater, Parameters, Helminths Eggs, Heavy Metals, Removal Efficiency

Chapter 1

1. Introduction

An average 70% of the surface of planet earth has been covered by water, rendering it as a vital component for sustenance of people as well as the health of the environmental systems. However, a non-sustainable consumption of freshwater resources, urbanization, elevation in population, and the drift in industrial development had resulted in environmental deterioration mainly by the ejection of semi treated or non-treated wastewater into aquatic systems (Bryant et al., 2018; Zhang et al., 2010). As a result, owing to the scarcity of clean water and inadequate sanitation, the idea of wastewater-to-resource is becoming increasingly popular among engineers and researchers involved in wastewater treatment knowledge. This problem, however, affects biodiversity conservation, environmental stability, health care and food security (Barman et al., 2015).

Water pollution results in various problems globally, including sanitation supplies, drinking water supply, and biotic species survival. Indirect pollution refers to contaminant's addition in the drinking water supply from ground or may be from the atmosphere through rainwater. Direct water pollution refers to the discharge of pollutants from factories, sewage treatment plants, refineries. Some general pollutants found in water include heavy metals, organic matter, nutrients, and pathogens. However, release of pollutants from industrial and domestic sources has harmful effects on aquatic ecosystem as this may result in deposition of high amount of organic matter, and nutrients leading to eutrophication (Song et al., 2019).

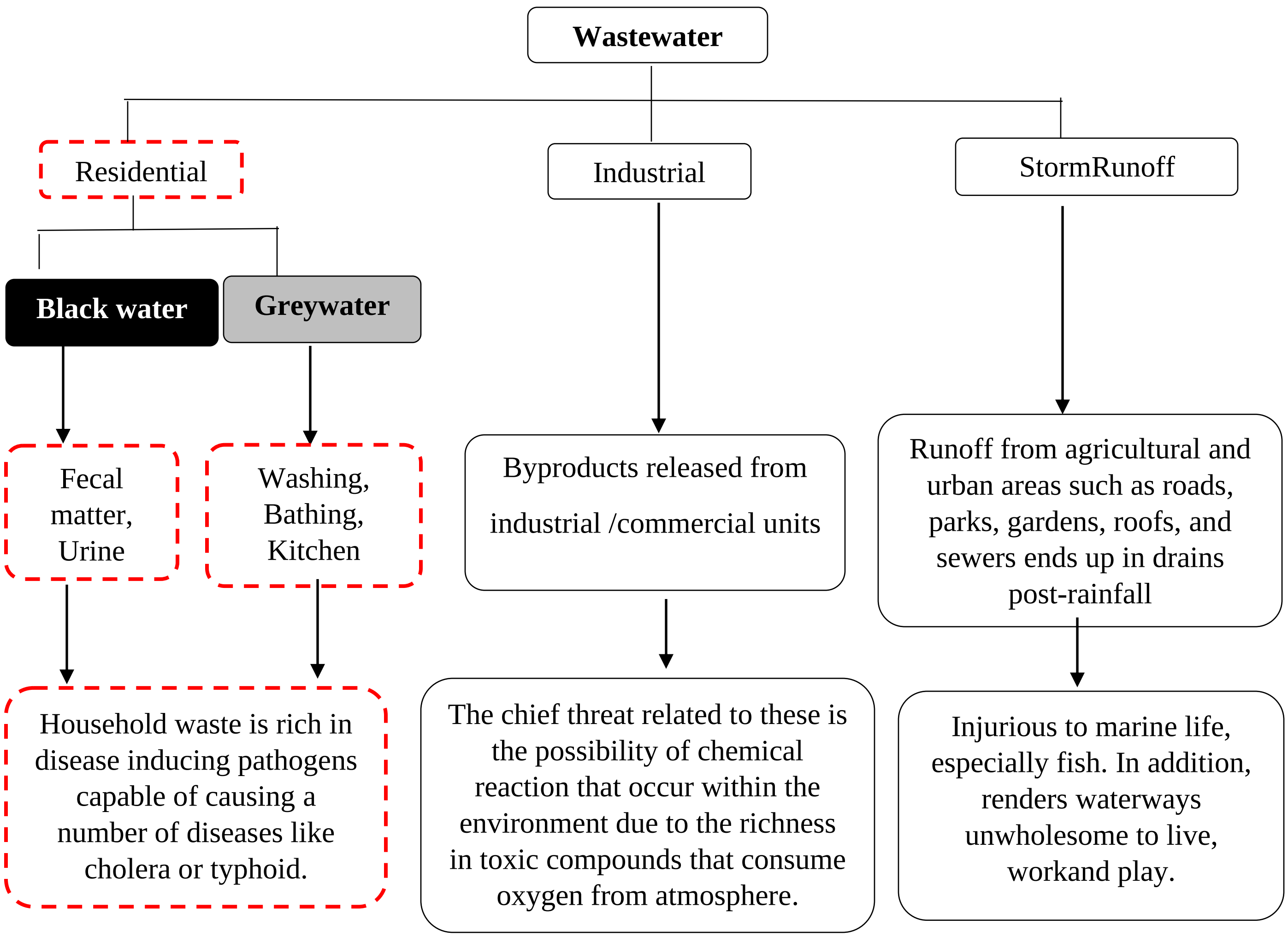

Figure 1: Types, Sources, and Effects of Wastewater

1.1.Wastewater situation in Pakistan

Lesser developed and developing countries of the world are the ones the most impacted by the issue related to water because of the limited resources and poor monitoring and implementation of NEQs for discharged wastewater. Polluted water is the root cause of disease in Pakistan (i.e., 60% of infectious diseases are waterborne). According to WHO report in 2012, around 7.8 billion people of the earth that is 1/10th of the total public still depends upon unhygienic water in 2010 and about 2500 million humans in the world still has the least approach to the modern sanitation system (Orebiyi & Awomeso, 2008).

The per head availability of water for the citizens of Pakistan at the time of partition was 5,600 m^3, however, the number has dramatically reduced to about over 406 percent from 5620 m^3 in 1951 to

1038 m^{3}in 2010. If the present trend continues, expected drop to an alarming level of 575 ft^{3}in 2050 forcing us into a transition from being a “water stressed” country to a “water scarce” one (Murtaza et al, 2012).

Pakistan in National Sustainable Development Goals (SDGs) framework (MPDR, 2018) mentions to diminish the discharge of chemicals and all other wastes i.e., solid, liquid, or gaseous to atmosphere, soil, and water to avoid their harmful impacts on environment and human health by 2020. United Nation’s Member State on 25th September 2015, implemented Sustainable Development Agenda 2030, which comprised of 17 goals, and it also incorporates clean water and sanitation, good health and wellbeing, resource consumption and production.

Table 1: Domestic Wastewater Composition

(WWAP, 2017); (Mara, 2003); (Hussain et al., 2002); (Chin, 2006)

	Pollutants		**%age in Domestic Wastewater**	**Impacts**
Organics and Nutrients	Biodegradable organics	Proteins, Carbohydrates, Fats, etc.	70% of solids from which Proteins are 48%, Carbohydrates 15%, and Fats 7%	• Depletion of DO • Fish mortality • Unsuitable environment
	Stable organics	Phenols, Pesticides, Chlorinated Hydrocarbon	Mainly depend on community due to their less use	• Environmentally unsafe • Render wastewater incompatible for agriculture
Inorganics	Suspended solids	Volatile compounds, colloidal impurities, salts, grit, etc.	30% of solid part of wastewater	Cause salinity and adverse impacts i.e., • Phytotoxicity • Impacts on the permeability and composition of soil
	Dissolved solids			
	Heavy metals			
Pathogens	Viruses	Adenoviruses Hepatitis A Gastrointestinal, Viruses	Mainly depend on community	• Cause communicable diseases
	Bacteria	*Escherichia coli* *Salmonella typhi* *Shigella* spp.		
	Helminths Eggs	*Taenia Saginata, Ascaris lumbricoides, Schistosoma spp.*		

1.2. Wastewater Treatment Methods

Water as an asset is becoming gradually rarer in many developing countries. Traditional technologies used for treating wastewater are known to be an effective method for pollutants removal but due to the high construction and exploitation costs of the treatment systems, there is need for a more safe, robust, less costly domestic water treatment technology is direly needed (Li et al., 2017). Therefore, it needs effective strategy for water management and strict implementation and monitoring. Figure 2 describes the different treatment methods with their advantages and disadvantages.

Constructed wetland (CW) are one out of the most common yet economical treatment technologies used around the globe. CWs design develops the principles and properties of natural wetlands and provides improved water quality. Constructed wetlands are robust, energy efficient, cost effective, chemical free, and easy to operate wastewater treatment systems (Wang et al., 2019). Furthermore, wetlands have been found to have an insignificant impact on the air quality, as the polluted water within the water flows below the surface, avoiding odors from spreading into the surroundings (Nandakumar et al., 2019).

Specified CWs consists of macrophytes that are introduced into porous medium (i.e., gravel, sand) and the wastewater is allowed to run mainly horizontally under the surface of their bed. Horizontal flow constructed wetlands efficiently remove organic matter, suspended solids, and pathogenic micro-organism from sewage (Shved et al., 2014).

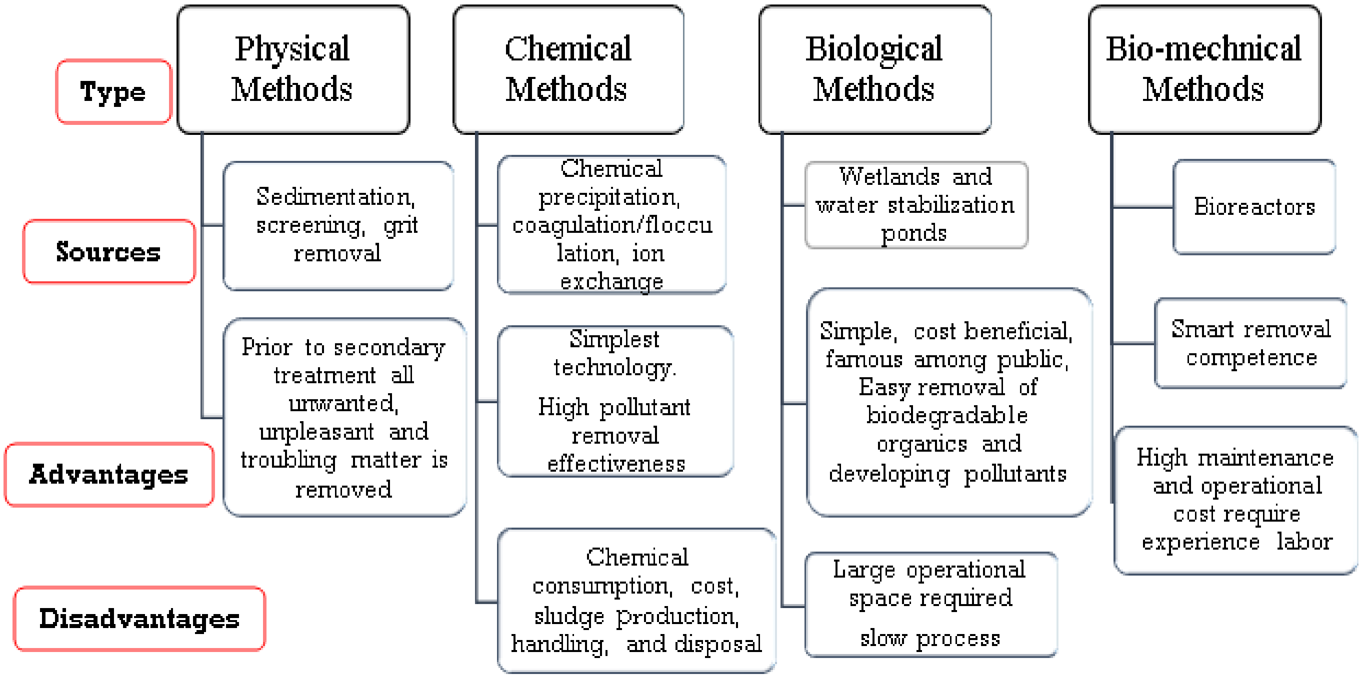

Figure 2: Wastewater Treatment Systems (Crini & Lichtfouse, 2019)

Utilization of treated and untreated wastewater entails threats associated with the presence of pathogens, which are of great concern. Among these pathogens, helminths eggs have the potential to cause diseases called "helminthiases" (Jiménez et al., 2016). As stated by the World Health Organization (WHO), a substantial emphasis on the existence of helminth eggs in wastewater has been placed and has been considered that these micro-organisms are the greatest microbiological health hazard coupled with the reuse of wastewater. Helminths eggs are mostly excreted out inside the excretory matter and are then spread though water, contaminated food, and soil having high reproductive capacity and resistance. They have complex and successful lifecycles. The health impacts caused by the hemlinth infections globally are leading to a multi-level extension in the research and implementation plans and agendas to control and reduce these ignored health issues. Resultantly, the WHO regulations have endorsed a limit of less than 1 helminth egg per liter of wastewater for the irrigational crops, sports fields and for public parks (da Rocha et al., 2016).

A main health issue which is ignored often is the existence of parasitic helminths eggs in wastewater along with the pathogens that cause disease. All over the world, it is assessed that around 2500 million individuals are infected with helminthiasis. The symptoms observed in these individuals commonly are toxic impacts on the intestines leading to its deterioration, damaged tissue, undernourishment, blood loss, irritation, diarrhea, and anemia primarily common in children of age 5-15 years influencing their lifestyle, physical and mental growth (King., 2019; Paruch., 2010).

About a thousand million individuals transfer chronic infections worldwide and endure each day from the outcomes of infection-associated pathogens. The most recent 2016 predominance assessments of the Global Burden of Disease (GBD) project (GBD, 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017) shows that around 0.8 billion cases of *ascariasis,*0.45 billion cases of *hookworm,*0.435 billion cases *of trichuriasis,*0.190 billion occurrences of the *schistosomiasis* disease, and 75 million incidents of food-borne illness *trematodiasis* around the world are present (King., 2019). Helminths eggs have the capability to persist in the environment for a longer period and have maximum capability for being transmitted through water by making them suitable contaminant depicters. To fulfill the increasing demand of water people are making the most of the resources of water that exist in the natural grounds which ultimately is leading to several environmental issues like ground water subsidence and ecosystem deterioration.

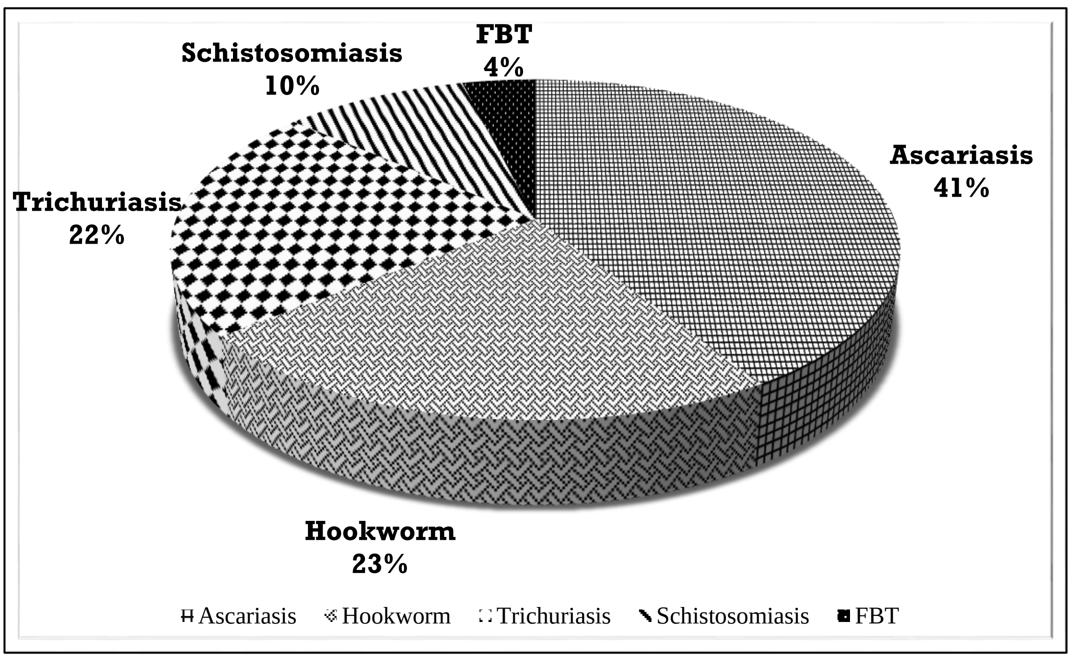

Figure3: Globally Helminths Prevalence Estimates

Recently, untreated sewage/industrial wastewater due to their widely usage is being released into surface bodies for disposal. The municipal wastewater is highly likely to contain this untreated water contributed by industries, residential users, and urban runoff. Due to the scarcity of water in this region, farmers are employing this wastewater to soak their vegetable lands in city conurbations. However, it may also have insignificant heavy metals which when uncontrolled, may be introduced by a variety of pathways as environmental contaminants and could lead to human beings and animals by the food chain (Nazir et al., 2015).

A few harmful heavy metal ions that have the capability to affect the human physical health are Arsenic (As), Chromium (Cr), Mercury (Hg), Cadmium (Cd), and Lead (Pb). These are added into the surroundings from a variety of sources such as chemical industries, textiles, leather factories, paints and pigments, mining, plastics, battery manufacturing, paper, and pulp industries etc. A few past incidents caused by the exposure and contamination of toxic wastewater being release in the waterbodies include: the Jintsu river *'Itai-Itai'* incident in Japan that resulted from cadmium contamination; and the very prominent *'Minamata'* disaster that occurred due to a careless exposure of methyl mercury. Once the high concentrations of these metallic ions are left to flow on the loose due to industrial operations, they might end up being in the water stream beyond the prescribed levels. The toxicity of these ions at low concentrations is exhibited at around 1.0 -10.0 mg/L and the main source of a toxic bio-adsorbents have been recognized as the agricultural activities and animal refuse matter (Chakraborty et al., 2020). The situation, therefore, is no less than alarming and thus desperately calls for prompt counteractive actions to be taken at all levels, to deal with the circumstances.

The concerns of continuously evolving pathogens are increasing continuously as they are resilient to water treatment with unsuitable spotting methods and technological failure. However, numerous traditional methodologies used for treating wastewater have been industrialized, however, the production of these in lesser developed and poor countries of the world is moderately high at present owing to their O&M cost. Besides these conventional techniques, a feasible alternative for treatment of wastewater in a lasting way is to execute integrated constructed wetland system (ICWs), a simple, efficient, and cost-effective method. ICWs are noteworthy in accomplishing an improved management of wastewater, including the elimination of suspended solids, heavy metals, as well as pathogens (Shingare et al., 2019).

1.3.Present Study

The current study was conducted for the treatment of wastewater from residential apartments, academic blocks, schools, institutes, and hostels in terms of different heavy metals, physicochemical parameters, and pathogens expulsion from ICWs at NUST Islamabad. Constructed wetlands concept appears to offer advantages mainly in developing countries for household wastewater treatment due to its operational simplicity, high pollutant removal efficiency, suitable construction, and low-cost.

1.4.Objectives

i. Assessing how efficiently ICWs work using various physicochemical parameters analysis.
ii. Quantifying and expulsion of Helminths eggs form wastewater, sediments, and plants.
iii. Analyzing relative efficiency of heavy metals uptake by water, sediments, and plants.

Chapter 2

2. Literature Review

The Max Planck Institute of Germany owns the origination of the idea and concept of Constructed Wetland system (ICWs) worldwide in the early 1990s. The idea was introduced to reach aims such as the improvement of wastewater treatment, and enhancement of the ecological and other wetland environment benefits by re-establishing wetland framework (Scholz et al., 2007; Harrington et al., 2005). Afterwards, in United States (US), the research work implementation was accelerated where a few frameworks were introduced primarily for domestic wastewater treatment as well as for agricultural wastes, industrial wastewater, and mining wastes (El-Refaie., 2010).

According to International Union for the Conservation of Nature and Natural Resources (IUCN), wetlands have been defined as "areas such as swamps, ponds, peat, temporary, permanent, natural, artificial, stagnant or flowing water. These also include estuaries and marine waters with a maximum depth of 6 meters at the time of low tide" (Nikolić et al., 2009).

Pros and cons of the treatment of domestic wastewater achieved by constructed wetland have been shown in the subsequent figure 4.

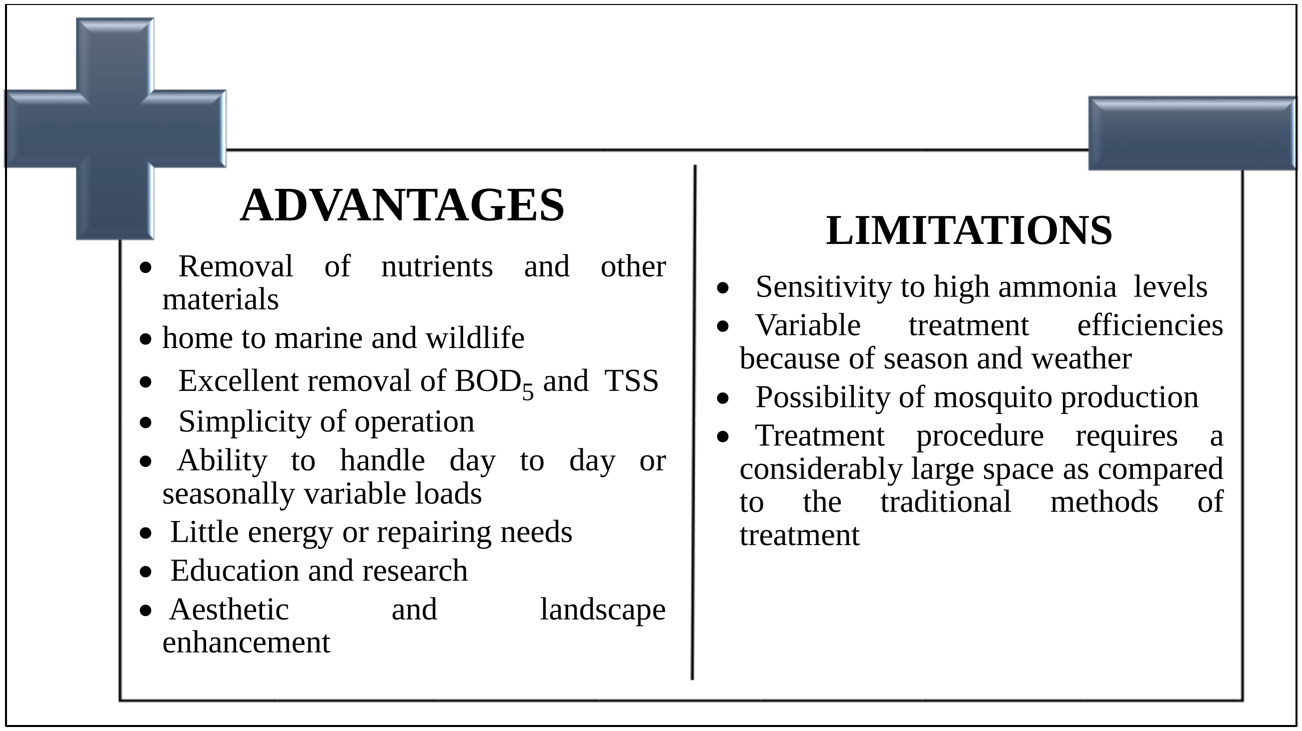

Figure 4: Advantages and Disadvantages of Constructed Wetland

2.1 Classification of CWs

Considering the present stage of technological development, there are three types of wetlands that are being used extensively:

2.1.1 Free water surface FWS wetlands are like natural marshes which have areas of open water like channels or basins, with soil, emergent and floating vegetation. They always fascinate a range of wildlife and offer habitat benefits like natural wetlands (Nikolić et al., 2009).

2.1.2 Horizontal subsurface flow (HSSF) wetlands are different from FWS wetlands as it consists of a sealed basin in which the wastewater flows horizontally below the bed plane from the starting part of the duct towards the end of it. The substrate of these is composed of a lined gravel bed where sprouting vegetation has been planted. As the wastewater flows subsequently from the gravel media and then around the roots and rhizomes of these plants, it is treated. Thus, the wastewater in HSSF-CWs is not uncovered during the entire procedure for its treatment; therefore, it causes less problems of public exposure, odor, mosquitoes, and insects. In addition, the risk of disease-causing agents (pathogens) to be exposed is also minimized (Yang et al., 2001).

2.1.3 Vertical flow (VF) wetlands may have the ability to dispense the water along its vertical path to pass through the filter media through the gravel bed or sand planted with emergent vegetation. The wastewater treatment is achieved when it seeps through the plant root area. VF systems contain a higher capability to transport oxygen and thus, present improved setting for carrying out nitrification (Kadlec et al., 2008).

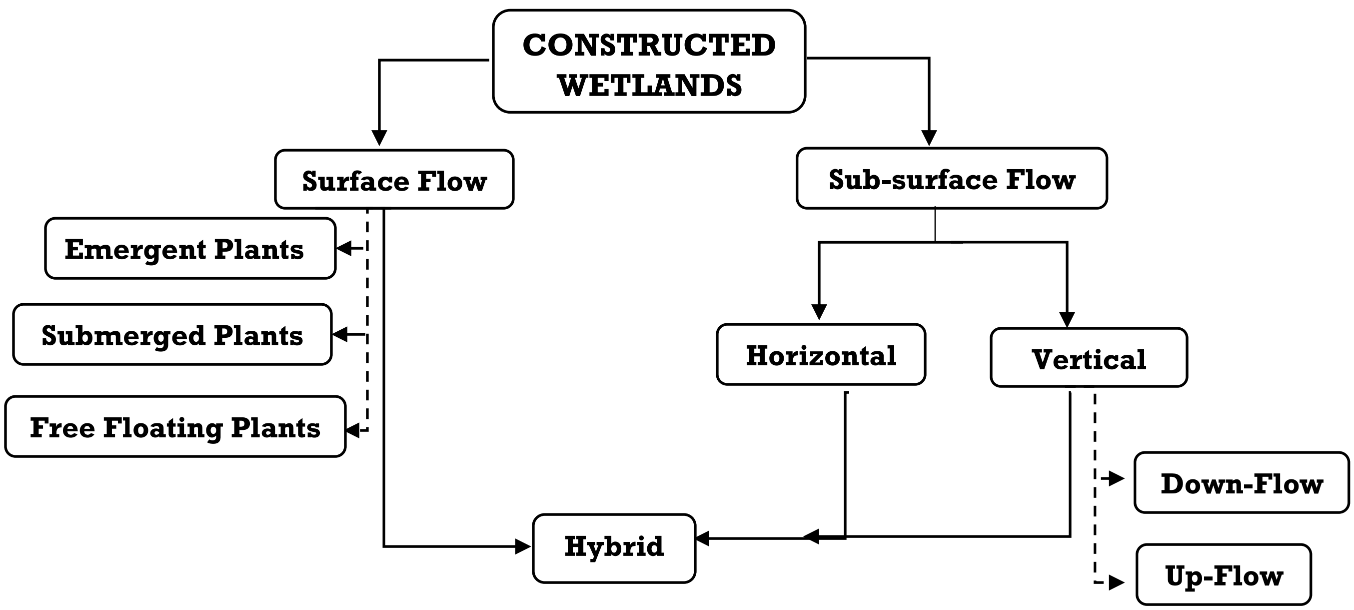

Figure 5: Basic types of Constructed Wetlands

2.2. Integrated Constructed Wetland Macrophytes

Macrophytes are considered as the most important biological constituent of wetlands depending on the types of wastewater treatment (i.e., Agricultural wastewater, domestic wastewater, or storm runoff) and climatic conditions of the wetland chosen area. Selected CW plants must have the capacity to endure overflow and to acclimatize relatively elevated pollutants concentration. However, for the treatment of both storm water and wastewater, native or local species may be utilized as they are adapted to soil, climatic conditions, and surrounding animal and plant communities (Achak et al., 2019).

Macrophytes perform various functions: prevent substrate clogging, encourage alluviation of suspended solids, provide stability, reduce chances of resuspension and erosion, facilitate filtration, extend the retention period for the surface water, promote microbial growth, and boost a reduced nutrient uptake and storage (Yang et al., 2020).

CWs plants have rich rhizomes and roots which help to remove nutrients and contaminants, tolerant to high organic loadings by acting as a storage space for microorganisms and helping in water purification. However, pathogen removal with the help of macrophytes is mainly due to the presence of antibacterial properties of plants. Thus, phytoremediation studies demonstrates that the purification effectiveness of wastewater through macrophytes are feasible, spontaneous, and remarkable (Aziz et al., 2015).

2.3. Supporting media or substrate

Wetland substrates not only possess the ability to sustain the vegetation growth physically, rather assist in transformations including biochemical and chemical side by side too. These also allocate sites for storing removed pollutants such as filtration, sorption, and sedimentation. Substrates include soil, sand, gravel, and organic materials (Wang and Zhang, 2012).

Constructed wetlands that are prone to greater reception of water rich in nutrients, for instance domestic or agricultural wastewaters are constructed with sand or gravel. They are often built with infertile site soils, and organic modifications, such as compost, leaf litter, or sewage sludge, incorporated into the substrate. This makeup of these wetlands acts as attachment planes for micro-organisms and microbial processes (Nandakumar et al., 2019; Saeed and Sun, 2012).

2.4. Recent studies in developing countries

A study was performed in Delhi Technological University campus to examine the efficiency of *brachiaria*-based constructed wetland for the removal of phosphorous as well as nitrogen in varied seasonal periods. However, the results show maximum phosphate average removal escalated from 55% in winter to 78% in spring, 80% in autumn, and 85% in summer as compared to nitrogen. The added benefit of using B. *mutica* based wetland system in terms of nutrient removal is that B. *mutic*a is rich in terms of the nutrients and can only be used as fodder for the livestock (Nandakumar et al., 2019).

Similar study was performed to demonstrate the plant contribution *(Phragmites australis, Iris pseudacorus, and Hyacinth pink*) for various pollutant removal (NO^{3-}-N, COD, NH^{4+}-N, and PO_4^{3-}-P) from CW-MFCs compared to conventional CWs. It was noted that, CW-MFC planted with *Iris* *pseudacorus* depicts high removal efficiencies of COD, NO^{3-} -N, $PO^4{}_{3-}$-P and NH^{4+} -N, by 46.29%, 71.5 %, and 96.60%, compared with the unplanted CW-MFC (Yang et al., 2020).

Helminths eggs identification and quantification in wastewater samples, a stepwise system was generated in MATLAB, that showed consistent outcomes. i.e., *Trichuris trichiura, Ascaris lumbricoides -fertile or unfertile, Toxocaracanis, Hymenolepisdiminuta, Hymenolepis nana, Taenia saginata,* and *Schistosoma mansoni*. The purpose of this system usage is minimal costs and time, uniform principal method that has the effectiveness of use for the recognition of helminth eggs that diminishes the process doubtfulness in the program. Additionally, it gives the improved ease of classifying species and identifying them based on their texture and morphologic features. However, it was concluded that the system only requires trained technicians for sample preparation and visualization (Jiménez et al., 2016).

A pilot level CW was performed in Taiwan to assess its performance efficiency. The results demonstrate better treatment efficiency from the month of Apr - Oct with removal rate of NH_3-Nfrom 78–100%, COD ranged from 13–51% of and orthophosphate reveals 52–85%. However, from Nov, the treatment efficiency decreased for orthophosphate up to 13% and NH_3-N up to 16%. It was concluded that the constructed wetland macrophytes display a huge pressure on the treatment efficiency of the wastewater (Jing et al., 2001).

Chapter 3

3. Materials and Methods

Methodology designed to achieve objectives of research were based on following phases.

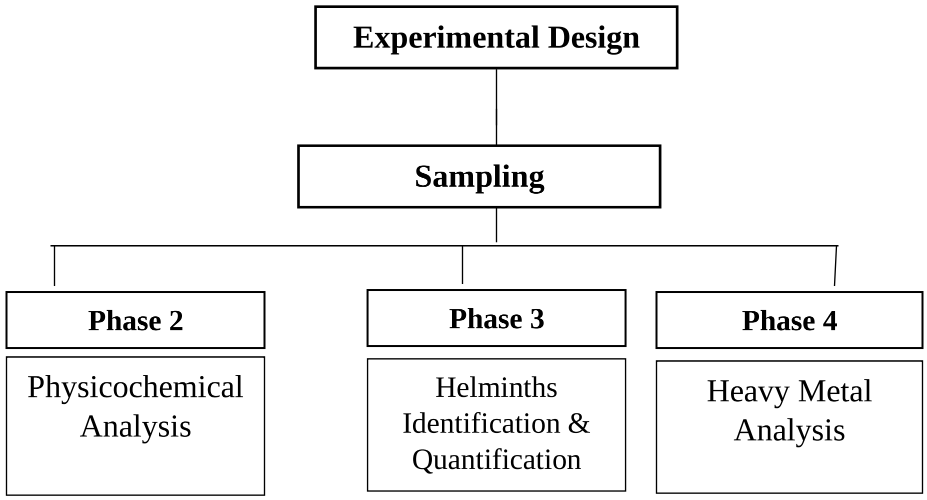

Figure 6: Methodology Flow Chart

3.1. Study Site

ICW study was conducted at NUST, H-12 Islamabad for urban wastewater treatment produced from residential apartments, institutes, offices, study blocks and hostels having 50 to 75 thousand Gallons per day treatment capacity.

ICWs, a biological wastewater treatment system, was funded by UNESCO. The layout of ICW comprises sedimentation tank (ST) having 35 feet, 12 feet, and 6 feet length, width, and depth respectively and here first pretreatment of wastewater takes place. After that, wastewater gets treated further in 8 ponds, however, Table 2 explain the characteristics and dimensions of ponds in detail. The further improvement in effluent quality from the ICWs was done through FILTER technique (Filter and Irrigated Cropping for Land Treatment and Effluent Reuse).

Figure 7: Integrated Constructed Wetland System (ICWs), NUST Islamabad

A graphic representation of Integrated Constructed Wetland System (wastewater treatment facility) developed at NUST has been depicted in Figure 8.

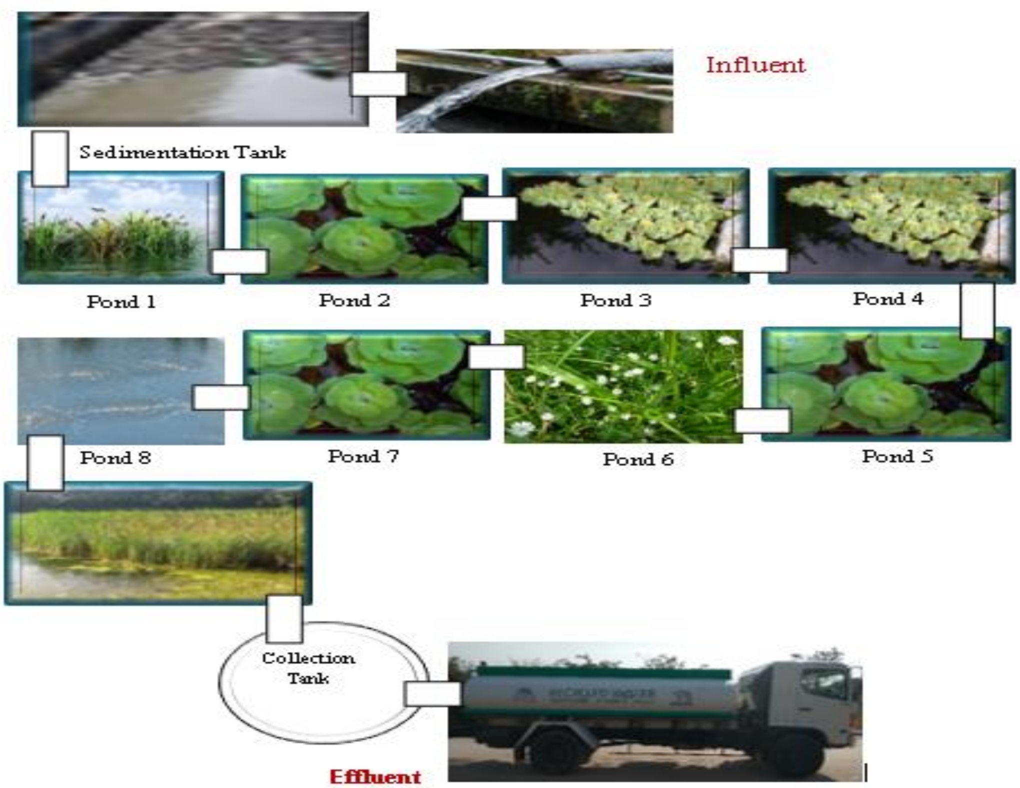

Figure 8: Schematic Diagram of Wastewater Treatment Facility

Table 2: Description & Characteristics of ICW Ponds

Ponds	Characteristics	Description
Influent	ST	Sludge removal further used as a fertilizer
P1	*Typha latifolia*	Great persistent grasses which are native to temperate and tropical areas (Vymazal, 2011). 15 plants approximately per m^2 are planted
P2	*Pistia stratiotes* (Water Lettuce)	Unbranched roots with light greenish-yellow shell-like plant (Pott & Pott, 2002). 10 plants approximately per m^2 are planted
P3	*Centella asiatica* (Penny Wort)	Efficient removal of pollutants in summer which drastically decrease its efficiency up to 50% in winters (Li. et al., 2018). 20 plants approximately per m^2 are planted
P4	*Centella asiatica* (Penny Wort)	20 plants approximately per m^2 are planted
P5	*Pistia stratiotes* (Water Lettuce)	Natural settling, aquatic, and sediment bacterial community act as removal mechanisms
P6	Stellaria Graminea	Natural settling, aquatic, and sediment bacterial community act as removal mechanisms.
P7	*Pistia stratiotes* (Water Lettuce)	10 plants approximately per m^2 are planted
P8	-------	Aeration system to improve O_2 level
Effluent	Collection Pond	Used for horticultural purposes

3.2. Sampling

Sterile glass bottles were used for the collection of samples that were previously rinsed with detergent properly and washed with distilled water. Bottles were then dried inside an oven at 106°C for about an hour. From September 2019 to February 2020, samples were collected twice a month from ICWs for physicochemical analysis. The collected samples were immediately shifted to Environmental Microbiology Teaching laboratory – IESE to perform further analysis of physicochemical parameters. APHA, 2017 standard procedure were followed for analysis of water and wastewater.

Figure 9: Wastewater Samples

3.3. Water Quality Parameters Analysis

3.3.1. Physicochemical parameters

Following twelve physicochemical parameters were performed to monitor the quality of water from ICWs and all the methods of analysis along with instruments are mentioned in Table 3.

	Table 3: Characterization of Physicochemical Parameters			
Onsite Analysis	• **pH** HACH 156 pH meter	• **Chemical Oxygen Demand** COD Digester	**(mg/L)**	Laboratory Analysis
	• **Temperature (^oC)** Laboratory Method-HACH	• **Total Dissolved Solids,** • **Total Suspended Solids** Gravimetric Dried Method		
	• **Electrical Conductivity(µS/cm)** Potentiometric Method - Conductivity Meter	• **TKN** Kjeldahl Apparatus		
	• **Dissolved Oxygen (mg/L)** Crison Oxi 45 DO meter	• **Turbidity (NTU)** Turbidity Meter 2100P		
		• **NO_3-N, NO_2-N, PO_4^{3-}-P** UV -Spectrophotometer Colorimetric Method		

3.4. Acquisition of Meteorological Parameters

Meteorological data study of 6 months from Sep 2019 to Feb 2020, was obtained from US-CASE, NUST. The data includes ambient temperature, wind speed, rainfall, and Direct Normal Irradiance (DNI) to find out the influence of such parameters on quality of water.

3.5. Identification and Counting of Helminths Eggs in Wastewater

For helminths identification known volume of treated and raw wastewater samples were collected and those samples were further left for settling for about 3 hours. Approximately 90% of the supernatant was removed by pouring it off after the process of settling or sedimentation. Larger particles were removed through sieve and helminths eggs were also removed by sieve with pore size of 170-180 µm and then both rinse and filtered sample were again left for settling for about 3 hours. Further, again supernatant was removed, and remaining sediments were transferred in centrifuge tubes and centrifuged for 20 minutes at 4000 rpm. After all the supernatant removal, zinc sulphate solution was added in sample and using vortex mixer solution was mixed thoroughly until homogenize solution was formed. Again, sample was centrifuged for 20 mins at 4000 rpm then equal volume of acetoacetic buffer solution and ethyl acetate were added to sample and centrifuged, after that in centrifuge tube three separable layers were observed. Total volume was measured, and the rest of supernatant was removed. In the end, prepared samples were shifted to the slide using Pasteur pipette and slide was observed under microscope using magnification of 10X.

For identification and inventory of helminth eggs in wastewater, Parasitological, Bacteriological Techniques, as well as laboratory instruction manual was used (WHO, 1996).

For the calculation of no. of eggs per liter following equation was used

$$N = AX/PV$$

N = No. of eggs per liter of sample

A = Mean of counts from two or three slides/ No. of eggs counted in the McMaster slide

X = Final product volume in ml

P = McMaster slide volume (0.3 ml)

V = Volume of sample (Original) in Liters

3.6. Heavy Metal Analysis

For heavy metals analysis water, sediment and plants samples were collected and analysis were done through following procedure.

3.6.1. Sample Collection

Water Sample

For water samples, samples were collected from selected ponds of integrated constructed wetland (ICWs) and for the storage of samples plastic bottles were used. After sample collection, samples were filtered to remove any large particles and then $HNO_3$was added to preserve the concentration of heavy metals in water.

Sediment Sample

Sediment samples were collected in zip lock bags, after collection samples were dried so that samples can be further converted into fine powder. After drying, sediments were fine powdered and sieved. From powdered sample, about 1 to 2 g of sample was taken and acid digestion was done using HCL and HNO_3. Digested sample was then cooled down at room temperature and filtered. Then filtered sample was diluted and stored in plastic bottles.

Plant Sample

For the collection of plant samples, stainless steel was used, and the purpose was to minimize the chances of contamination. Collected plant samples were rinsed properly with tap-water and again with distilled water. It is done to eliminate any remaining material that may be present on the surface of plants. However, plants samples were collected at peak time of seasonal growth. Likewise, sediment samples, plant samples were also collected in zip lock bags and same procedure was followed as mentioned earlier for sediment samples.

3.6.4. Sample Analysis

To find out the heavy metals in water, sediment and plant samples Atomic Absorption Spectrophotometer was used.

3.7. Statistical Analysis:

IBM-SPSS software was used for statistical analysis.

- Correlation among different parameters i.e., between meteorological and water quality parameters were observed to determine the relationship among all.
- Mean for duplicate values were computed and then standard deviation was applied.

4. Results and Discussion

Water quality is defined by different physicochemical parameters. However, these parameters may vary because of several other variables such as pollution type, water source, seasonal fluctuations, and adjacent human intervention.

4.1. Comparative analysis of climatic parameters effects on water quality parameters of treatment system

Data of three meteorological parameters for six months were obtained from US-Case, NUST. Parameters include ambient temperature, Direct Normal Irradiance (DNI), and wind speed. Effect of these parameters on water quality parameters is mentioned in detail below:

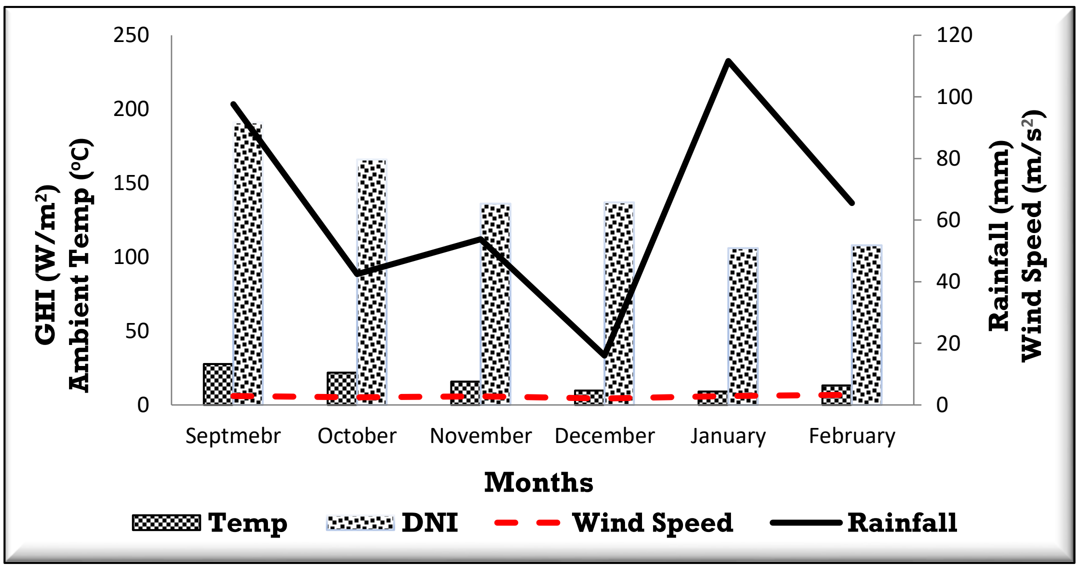

Figure 10: Climatic Parameters

- **Direct Normal Irradiance (DNI)** The quantity of radiations from sun that are collected by the surface per unit area which held perpendicular to the sunrays that come forming a straight linear trajectory of the sun at its present position in the sky is known as DNI.
- Thus, better photosynthetic activity by plants was observed in ICW due to increase of DNI which in turn results in uptake of nutrients and minimum discharge of decaying organic matter.

- **Wind** is the movement of air from higher pressure to lower pressure areas depending upon the fluctuation in air temperature (Wanninkhof, 2014). Lower wind speed enhances pollutants residence time and in turn results in lower dissolved oxygen concentration, affecting the photosynthesis process.

  Study conducted by wood and Chang in2006 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.
- Temperature is seen to present a visible effect on the pollutant removal efficiencies as well as total pollutants load of treatment systems as biomass, biodegradation and microbial community structure is extremely reliant on temperature fluctuations (Meng et al., 2014).

4.2. Analysis of Physicochemical Parameters

ICW design plays its role in anaerobic digestion in top 2 feet due to convection by wind, atmospheric diffusion, and plant roots with rhizosphere, whereas anaerobic digestion is from 3-7 feet on benthonic surface and thus plant involved mechanisms includes phytoextraction, phytodegradation, and rhizo-filtration. Every parameter: Temperature, pH, EC, DO, COD, TDS, TSS, and Helminth eggs fulfill the standards of agricultural reuse as mentioned below.

Table 4. National Environmental Quality Standards

Parameters	Units	Existing Standards for wastewater discharge	Revised Standards for wastewater discharge			International Agricultural reuse standards (Non-fodder crops)
			Inland waters	Sewage	Sea	
Temperature	°C	40	≥3	≥3	≥3	----
pH	-	6 - 10	6 - 9	6 - 9	6 - 9	6.5-8.5
COD	mg/L	150	150	400	400	<150
TSS	mg/L	150	200	400	200	<100
TDS	mg/L	3500	3500	3500	3500	---
EC	μS /cm	---	---	---	---	>2500 unacceptable
Helminths	egg/ L	---	---	---	---	<1
Reference		NEQS, 1995	NEQS, 2000			US-EPA, 2006 34

4.2.1. pH

pH as an important factor basically a measurement of hydrogen ions activity (Barman et al., 2015). It is an indicator of analyzing the widespread environmental condition. It affects various chemical and biological processes therefore it plays a major role in pond productivity (Sangeeta & Neha 2015). As depicted in Figure 11, pH was found to be alkaline throughout the analysis of samples which is ideal for immense diversity of microbes (Vymazal, 2013b; Parades et al., 2007).

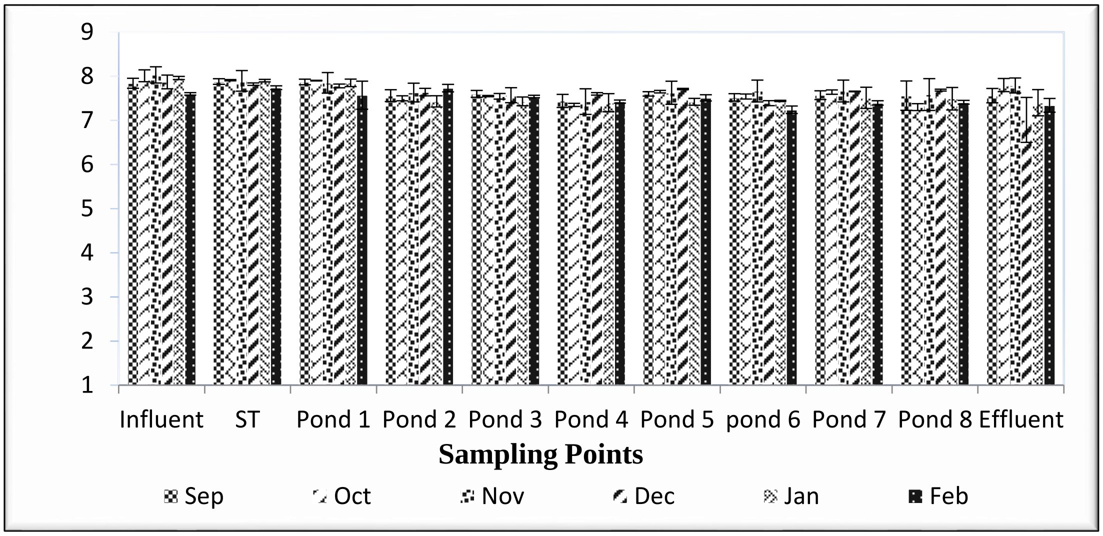

Figure 11: Variation in pH

4.2.2. Temperature

A natural feature influencing water quality and acting as a highly important parameter in working of aquatic system is known as Temperature. Various factors alter the temperature of ICW are daytime, season, vegetation, and TDS present in wastewater. In the current examination the seasonal analysis depicts a lowering trend in different months i.e., Sep – Feb that might be because of drop in organic matter concentration. The same kinds of results were reported by (Agrawal et al., 2018). Microbial degradation and plant mechanism activity is greatly influenced by moderate temperature (Oslandet al., 2018; Feher et al., 2017) while with the reduction in temperature degradation rate also slows down (Meng& Li, 2014).

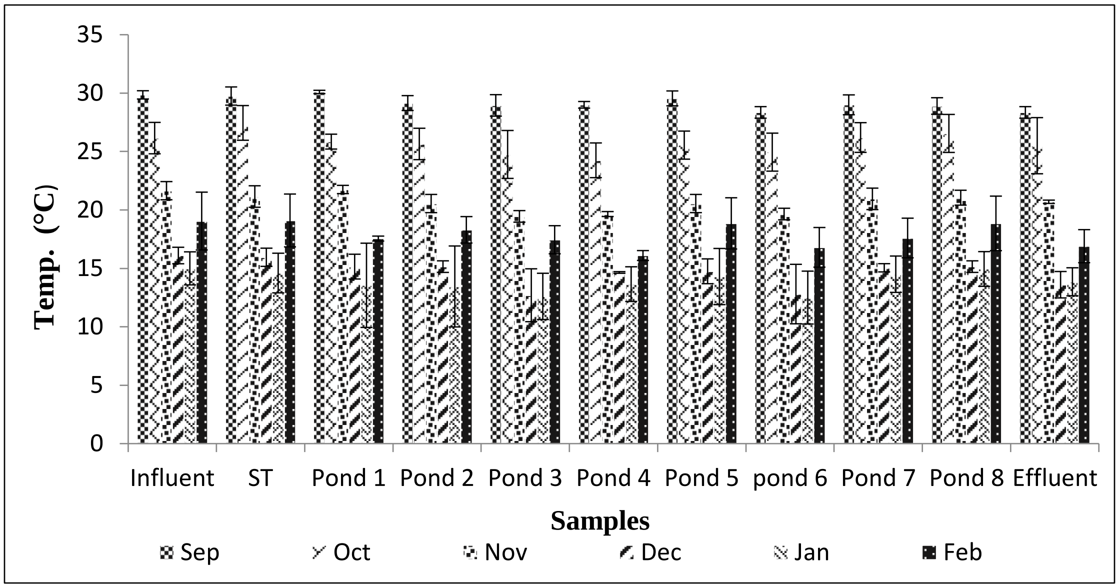

Figure 12: Variation in Temperature

4.2.3. Dissolved Oxygen (DO)

Many metabolic processes of aquatic organisms are regulated by presence of dissolved oxygen in water therefore it carries a great limnological importance. During the rainy and winter season the values of dissolved oxygen was noted high might be owing to reduced nearby temperature and least DO values in autumn (8.38 mg/L) might be because of higher rate of metabolic organisms (Barman et al., 2015). Thus, DO is also greatly enhanced by the roots of plants and diffusion through the air which in return enhance microbial degradation even in depth. Highest value was observed in Dec due to low temperature that enhances DO in water (Shen et al., 2019).

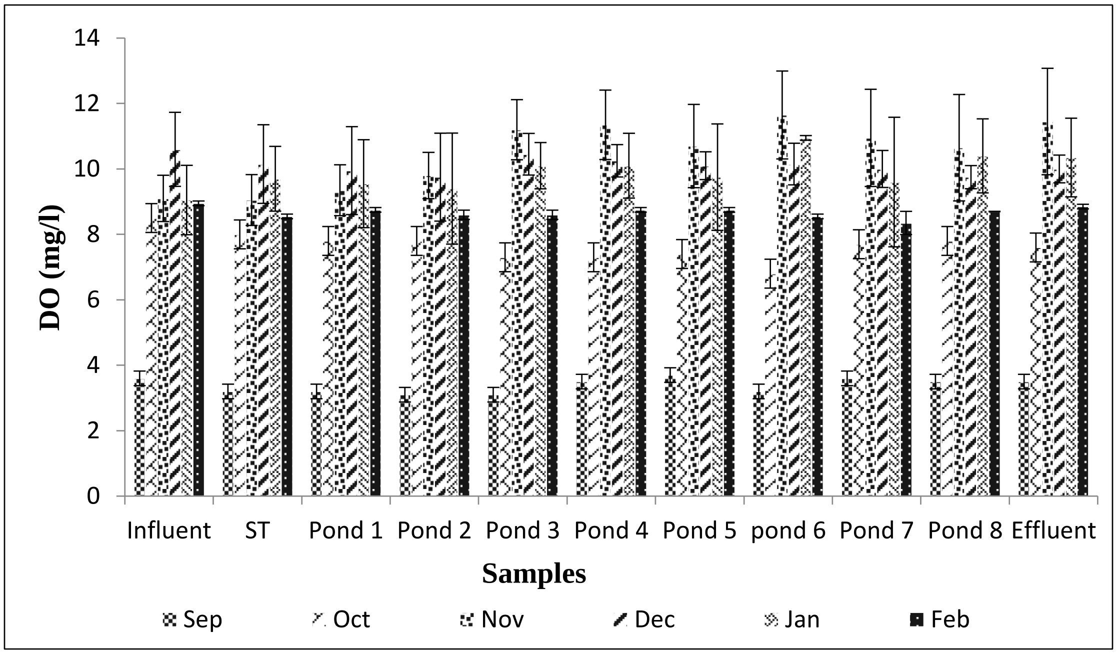

Figure 13: Variation in Dissolved Oxygen (DO)

4.2.4. Electrical Conductivity (EC)

Electrical Conductivity is an important parameter in analyzing the quality of water which shows the total concentration of ions dissolved (organic and inorganic) (Sangeeta & Neha., 2015). High EC leads to eutrophication, salinity problem and results to water pollution. Figure 14depictsa rising trend in the level of electrical conductivity within sampling points that might be caused by the lowering in the TDS concentration and the transformation of NO_3-N into (N_2) as the charged ions concentration lowers (Shamaet al., 2013).

EC and temperature relationship may be illustrated as when temperature is increased the solubility of minerals and other inorganic matter are also increased. Same kinds of results were reported by Agrawal et al., 2018.

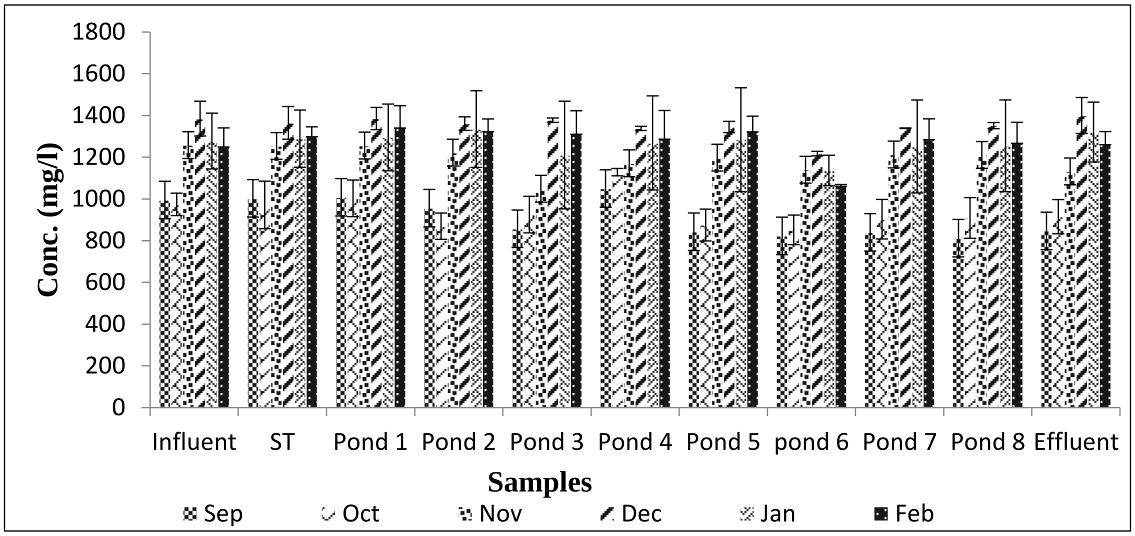

Figure 14: Variation in Electrical Conductivity (EC)

4.2.5. Turbidity

This factor in water is due to the colloidal and suspended substances that includes fine organic as well as inorganic matter, clay, silt, and another microscopic organism (Sangeeta & Neha., 2015). Figure 15indicates that in P4 there is high turbidity because of the attachment of suspended, tiny particles on roots and other plant debris whereas in collection tank lowest value was observed (de Jonge et al., 2014). Removal efficiency of turbidity of ICWs indicated was 90, 78, 73, 84, 83and 81% in September, October, November, December, January, and February, respectively.

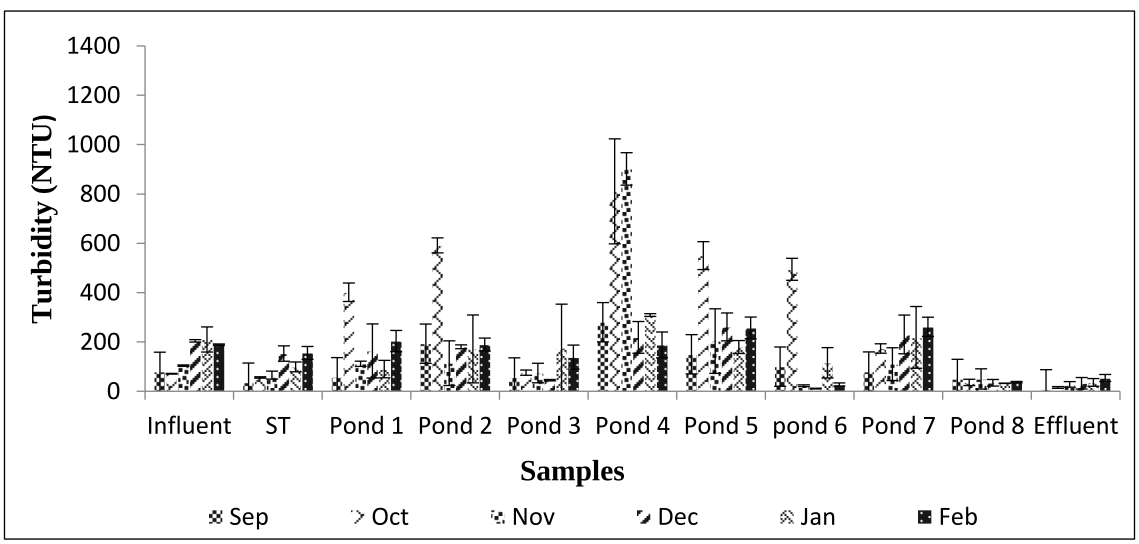

Figure 15: Variation in Turbidity

4.2.6. Total Dissolved Solids (TDS)

Estimation of mixed content of all organic and inorganic substances is known to be total dissolved solids. Greater amount of TDS causes eutrophication in water bodies which ultimately results to pollution. Figure 16depicts that in outlet (collection tank), there is less concentration of dissolved solids might be because of fewer solid accumulation of solid by the downfall in water speed as it moves from one pond to the other. It may also have occurred because of the uplift of dissolved solids by ICW plants present. Moreover, the value of TDS is in the range that is set by NEQS (Gitau & Kitur, 2016).

TDS removal efficiency in ICWs observed was 15, 38, 7, 9, 5 and 2% in September, October, November, December, January, and February, respectively.

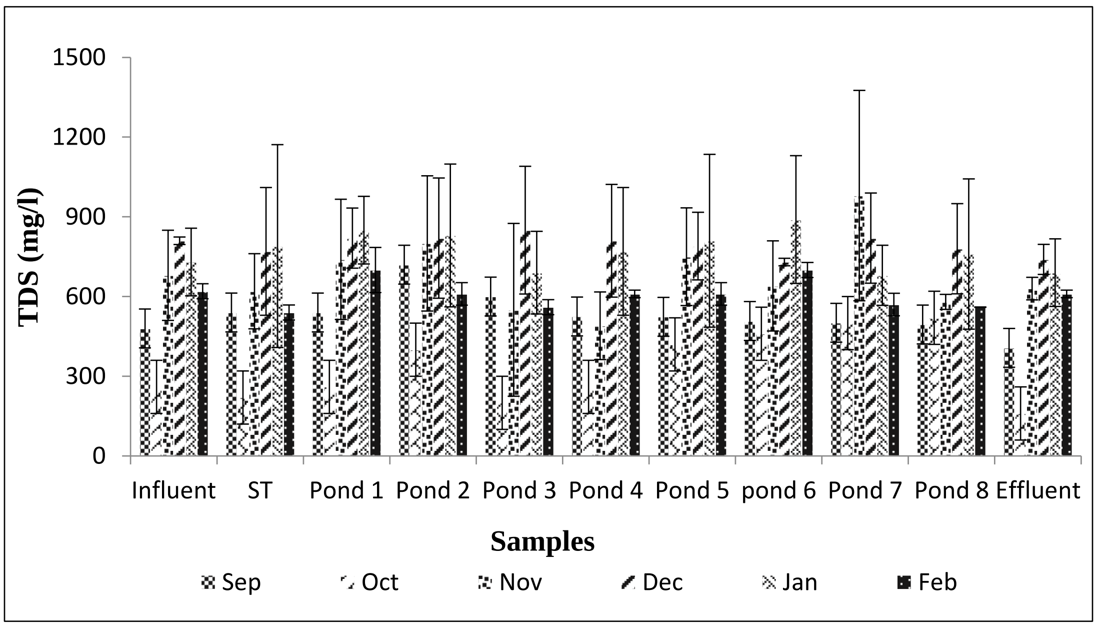

Figure 16: Variation in Total Dissolved Solids (TDS)

4.2.7. Total Suspended Solids

Suspended form of solids that remain in water and are contrary to the settling of matter is called TSS. Figure17 reveals that in Pond 3 and 4 there is an increase in suspended solids might be because of death and decay of plants results in rise of organic matter. Contrary to it, least concentration observed at collection tank may be because of wetlands plants that help in pace reduction of flowing water through various ponds thus resulting in suspended solids to settle at the surface of the water column

(Weerakon et al., 2016). The trend of TSS removal efficiency in ICWs observed is as follows 100% (October) > 96% (September) > 91% (November) >86% (December) >83% (January) and 82% (February).

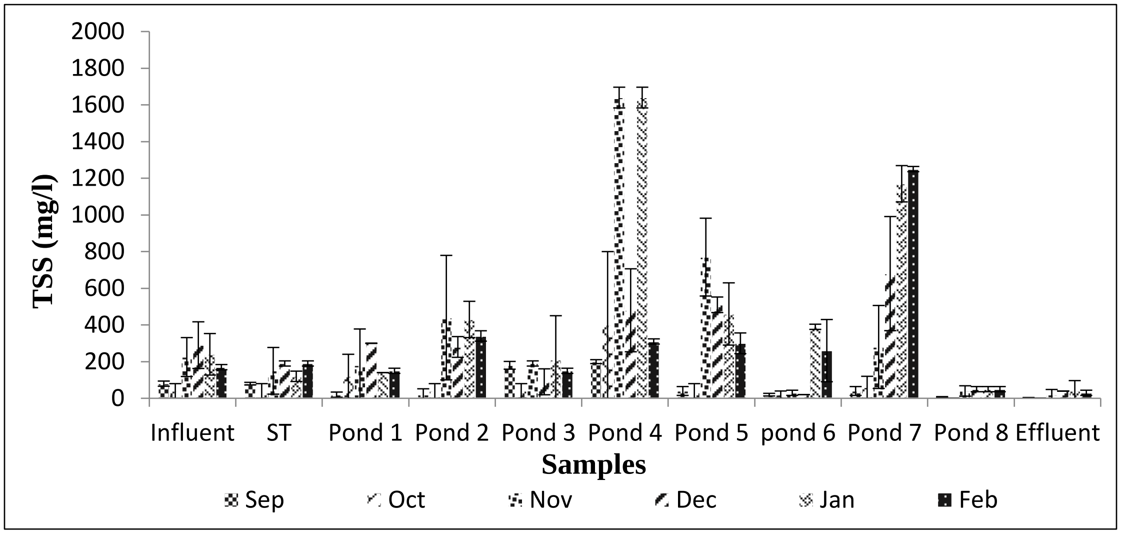

Figure 17: Variation in Total Suspended Solids (TSS)

4.2.8. Nitrate – Nitrogen (NO_3- N)

The presence of excess algal blooms in water body may be the result of greater concentration of Nitrate. Nitrates are impacting to fresh water mainly due to industrial waste, expulsion of sewage, and rubbish fertilizers, rotten plants, and cattle refuse from fields and farms. The standard seasonal mean for the sampling points was found to be within the permissible bounds (Barman et al., 2015). The removal efficiency of nitrate was 98, 93, 97, 34 and 92% in September, October, November, December, January, and February, respectively.

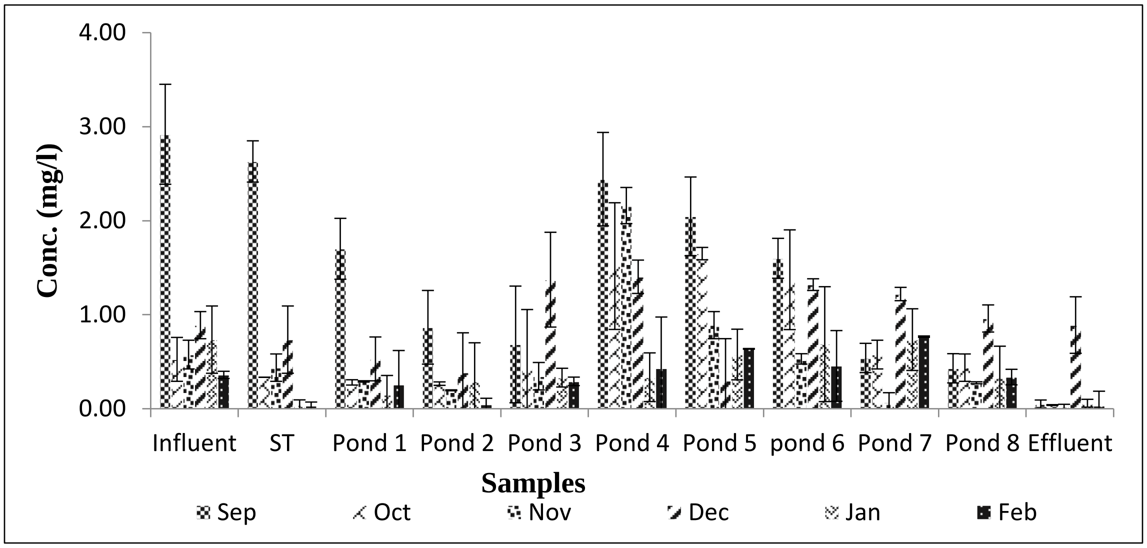

Figure 18: Variation in NO_3 - N

4.2.9. Nitrite-Nitrogen (NO_2 - N)

Nitrite in water is either due to reduction of nitrate or due to oxidation of ammonium compounds. Nitrite is unstable, as an intermediate stage in the nitrogen cycle (Sangeeta & Neha, 2015). Nitrite removal efficiency of the wetland system was recorded as 61, 66, 81, 42, 78, and 72% in September, October, November, December, January, and February, respectively.

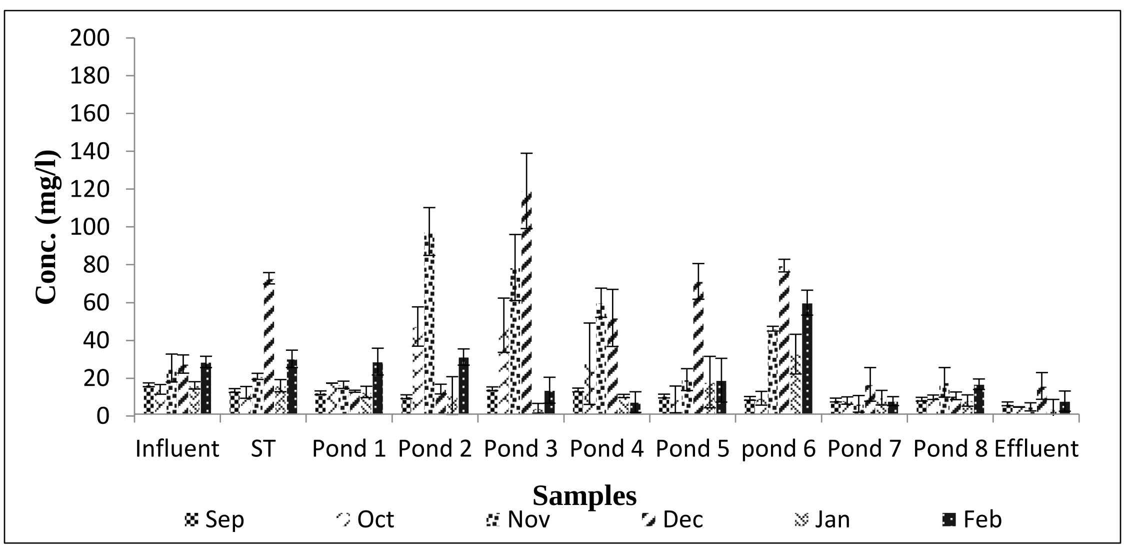

Figure 19: Variation in NO_2 - N

4.2.10. Total Kjeldahl Nitrogen (TKN)

TKN is a combination of ammonia and organic nitrogen which varied across several ponds and minimal values were noted in collection tank. Influent having increased organic nitrogen concentration in wastewater results in rise of TKN concentration in the few primary ponds probably owing its transformation to ammonium ions (He et al., 2018).

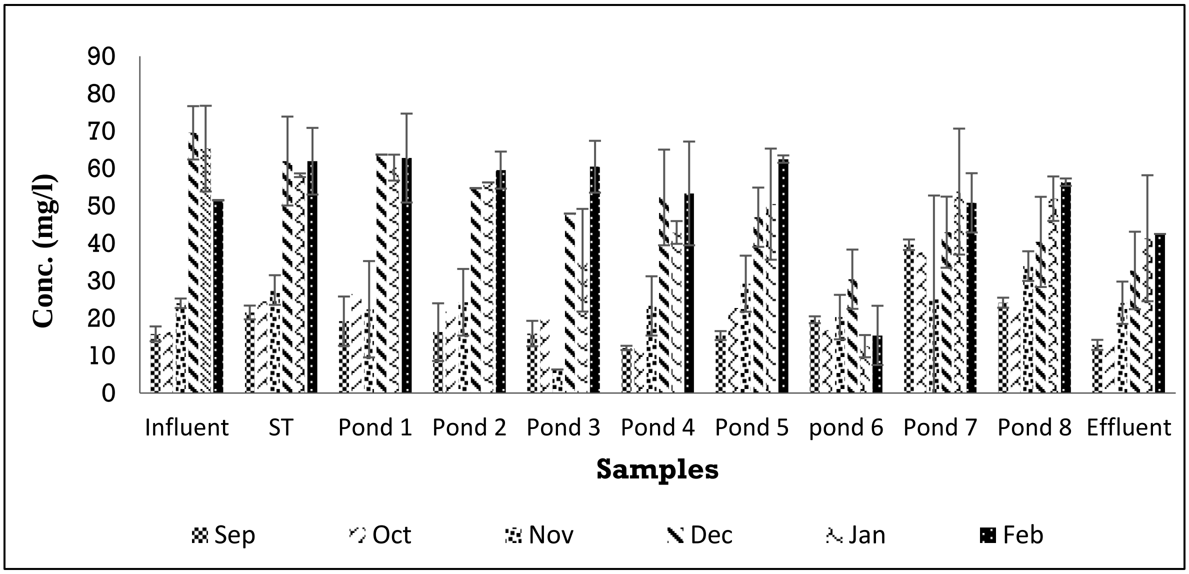

Figure 20: Variation in TKN

4.2.11. Phosphate – Phosphorous (PO_4^{3-}- P)

An important parameter that causes eutrophication is phosphate that leads to an extensive algal growth. In autumn high nutrients observed is caused by the lowering of water level which ultimately effects concentration and the discharge of nutrient while decomposition. Similar results were found by various workers i.e., highest phosphate in rainy season and least in the winter season (Gitau & Kinar., 2016). PO_4^{3-}- P removal efficiency of ICW was 79, 95, 82, 74, 53 and 70% in September, October, November, December, January, and February, respectively (Barman et al., 2015).

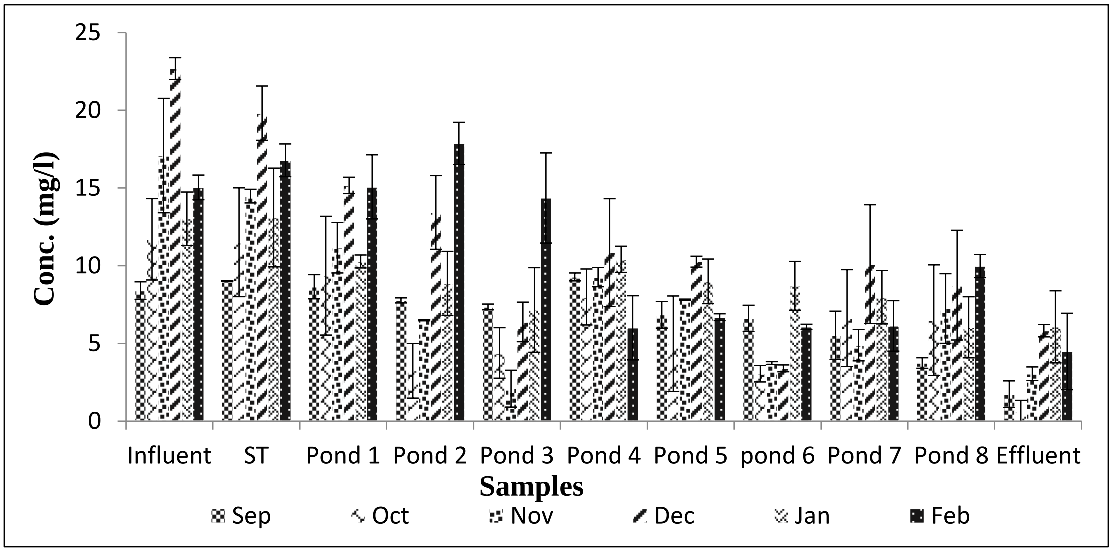

Figure 21: Variation inPO_4^{3-}- P

4.2.12. Chemical Oxygen Demand (COD)

Organic load and microbial activity greatly influence the COD removal efficiency. Removal efficiency of COD for both the system was lowest in winter due to the significant decrease in temperature (Mancilla et al., 2013). Due to the increase growth and establishment of plant removal of pollutants increases (Rai et al., 2013). In the season of winter, lower organic matter removal was observed by phytoremediation system because of decreased plant density. The removal efficiency was observed for the months Sep to be 79, Oct 76, Nov 78, Dec 77, Jan 64, and Feb was 77%.

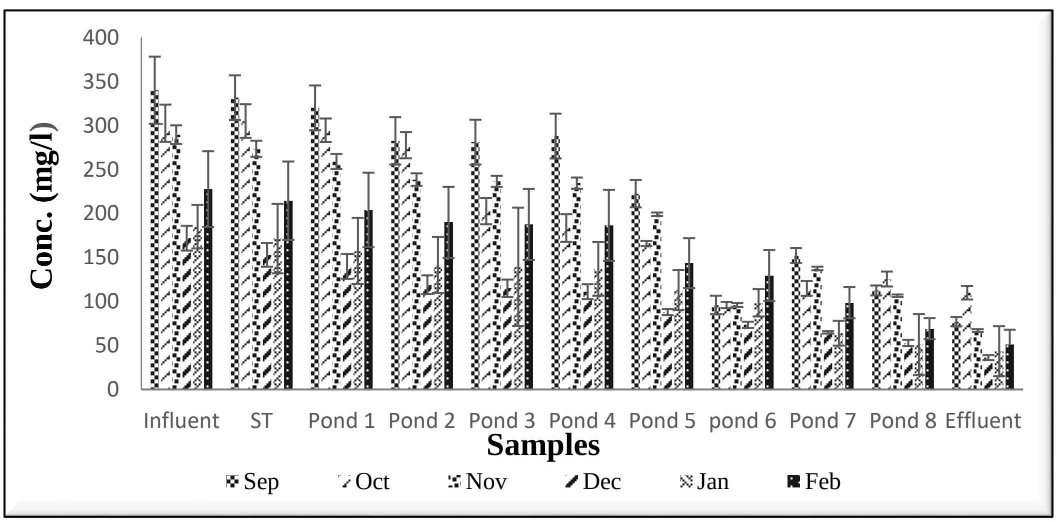

Figure 22: Variation in Chemical Oxygen Demand (COD)

At cold environments, seasonal influence on performance of constructed wetland may be important. Temperature and microbial activity are inter-linked. With decreasing temperature bacterial growth and metabolic rates were found to decrease. This examination that in winter the average removal efficiency gets less as compared to the rest of the year might be imputed to higher temperatures of wastewater maintained by the insulating layer. Therefore, it is interpreted that performance of this technology at minimal temperature is vigorous and strong (Wu et al., 2011).

4.3. Helminths Identification and Enumeration

Recommended limit for the eggs of helminth according to Agricultural reuse standards is <1 egg/Liter (Grego et al., 2018). In the samples of inlet wastewater, a diversity of helminths parasites was indicated when observed through microscope. Academia and residential were the major source of water, therefore the detected eggs of helminth in study were solely due to human origin (Anjonina et al., 2015). This elevation in the eggs of helminth is imputed to rise in temperature which led to the maximum count of microbes. Existence of the eggs of Helminths has been found to have a relationship with disease rate within community and to the size of community being served (Sabbhai et al., 2018). Most wide-spread helminths species of Phylum Trematode, Nematode and Cestode that were recognized have been displayed in Figure 23.

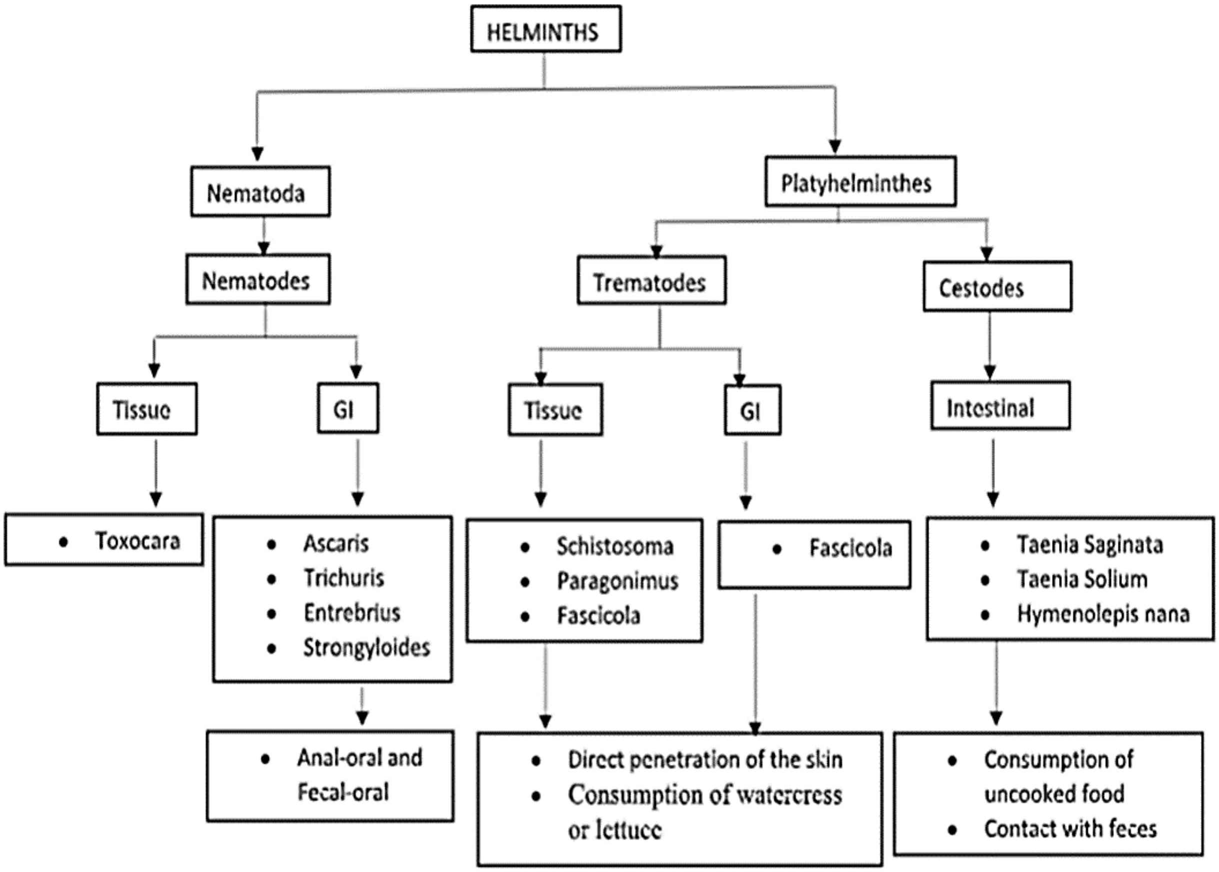

Figure 23: Presentation of the phyla, classes, and genera of Helminth eggs

4.3.1. Presence of Helminths eggs in Wastewater, Sediments and Plants

Figure 24 shows persistent depleting scenario in each pond of ICW. Sedimentation and stabilization on bed of ponds was the main cause for the removal of Helminths egg. Helminths eggs were found maximum in September, October, November, and December i.e., 100 % removal while lowest number of eggs were found in Jan and Feb i.e., 50 % removal. It may be due to a positive relation between the temperature and micro-organisms. High temp leads to high oxygen level in the rhizosphere, which tends to be discharged through the roots. Additionally, organic matter also leads to high growth rate of micro-organisms (Molleda et al., 2008).

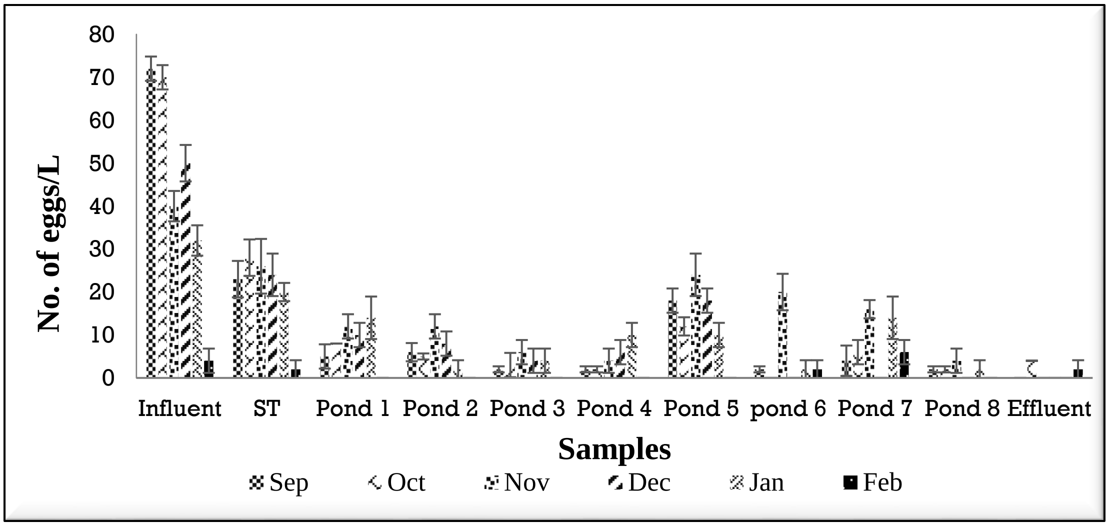

Figure: 24: Variation of Helminths eggs in Wastewater

Before reaching the aquifer, the Helminths egg concentration in wastewater is decreased by various orders of magnitude because of the wastewater infiltration through soil. Figure 25 shows that with the increase in depth of soil the concentration of Helminths egg is reduced (Cansigno et al 2013).

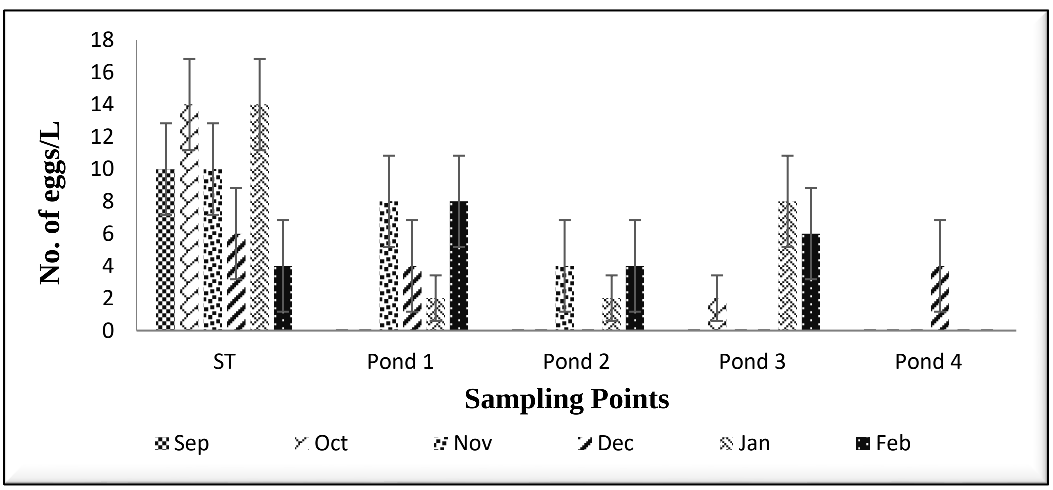

Figure 25: Variation of Helminths eggs in Sediments

Purification of water is greatly influenced by extensive root structure. Because of the large surface area provided by ICW plant the attachment of microbes to the root structure becomes easy and helps in parasite removal (Shingare et al., 2017).

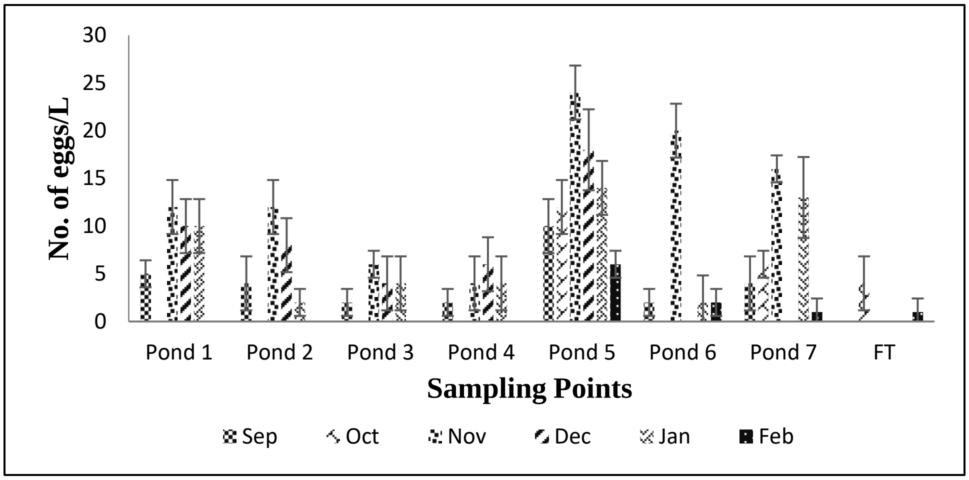

Figure 26: Variation of Helminths eggs in Plants

4.3.2. Helminths egg identification

The prevalence of these eggs in wastewater coupled with pathogenic agents needs to be considered as a wellbeing issue that is ignored most often. Most of the times these parasitic helminths eggs are released through faeces, and they intensify through soil, water, and contaminated food. It was observed that wastewater has the largest concentration of Helminths egg in contrast to sediment and plants.

i. Nematode

Considering size and appearance, several species of Nematodes were recognized. Identified species includes *Trichuris trichiura*, fertile and non-fertile *Ascaris lumbricoide*, and *Toxacara canis* (Chaoua et al., 2018).

The *Ascaris* nematode among the helminths egg is observed to be the most obstinate to treatment processes. *Ascaris* infects about one quarter of the world's population and due to the poor sanitation in developing countries this infection is more prevalent (Gupta et al., 2009; Cooper et al., 2018). Figure 31 depicts that *Ascaris Lumbriocoides* egg as compared to other helminth eggs i.e., *Toxocara canis*, and *Trichuris trichuria* showed higher resistance to adverse conditions like alkaline pH and temperature (Rocha et al., 2016).

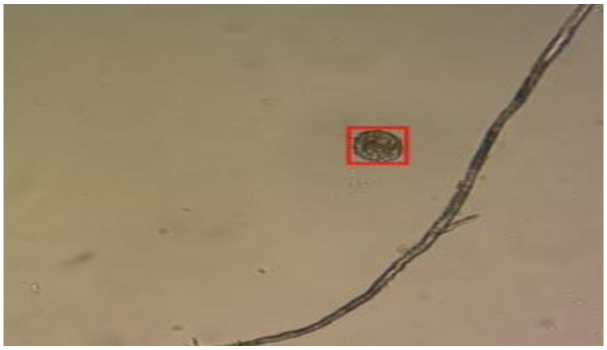

Figure 27: ***Fertile Ascaris lumbriocoides***

Figure 28: *Toxocara Canis*

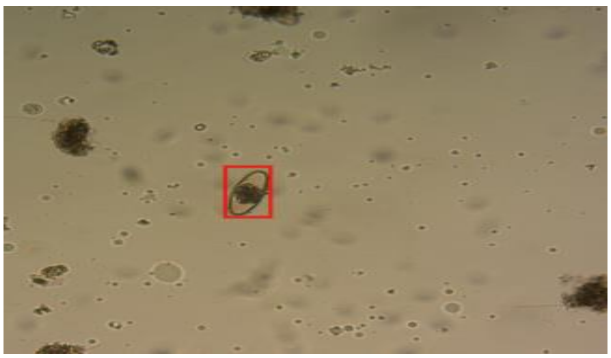

Figure 29: *Trichuris Trichuria*

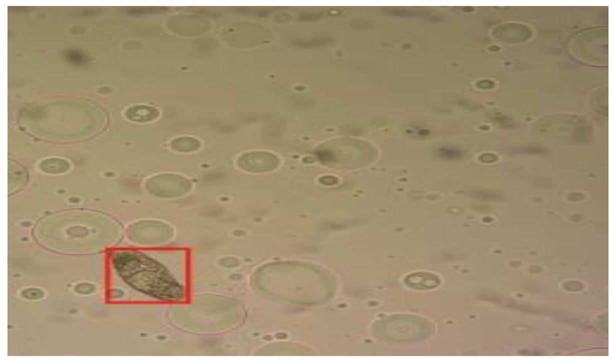

Figure 30: *Ascaris* Infertile

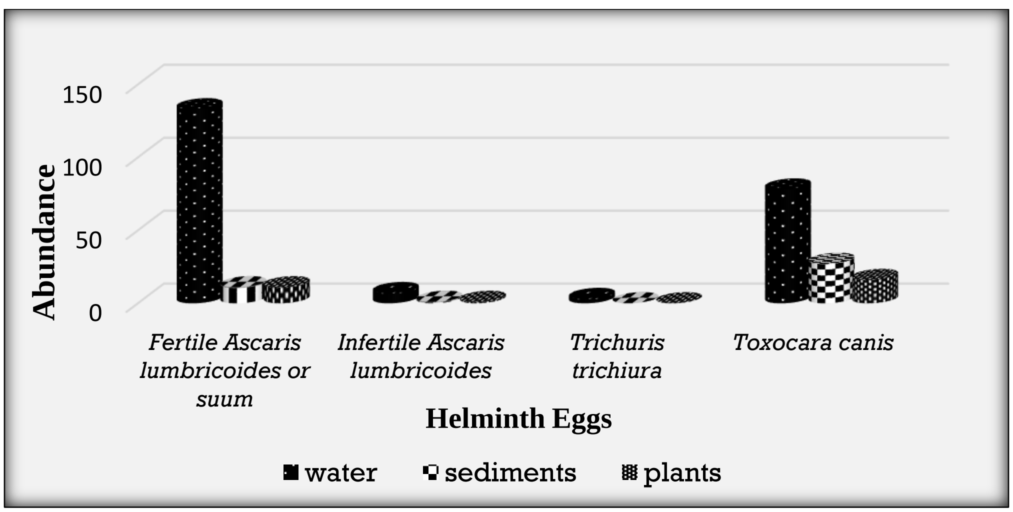

Figure 31: Nematode species abundance

ii. Trematode

Fasciola Hepatica and *Schistoso mamansoni*are among the recognized Trematode species. Figure 34 confirms a prevalence of Fasciola eggs species in wastewater followed by sediments and plants.

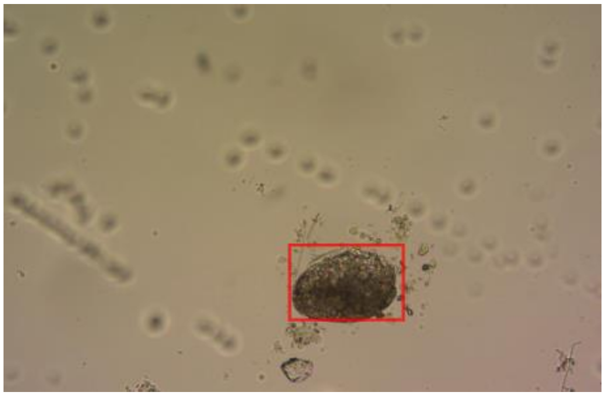

Figure 32: ***Fasciola Hepatica***

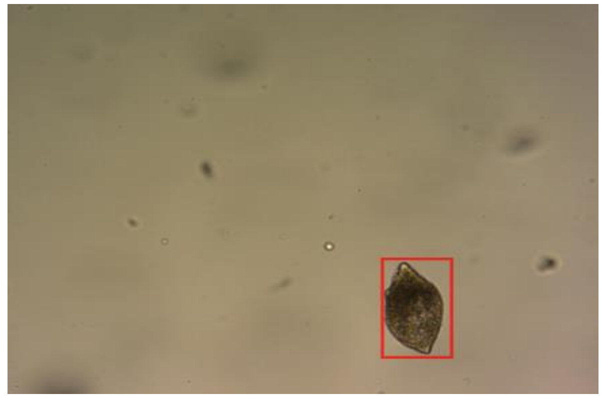

Figure 33: ***Schistosoma mansoni***

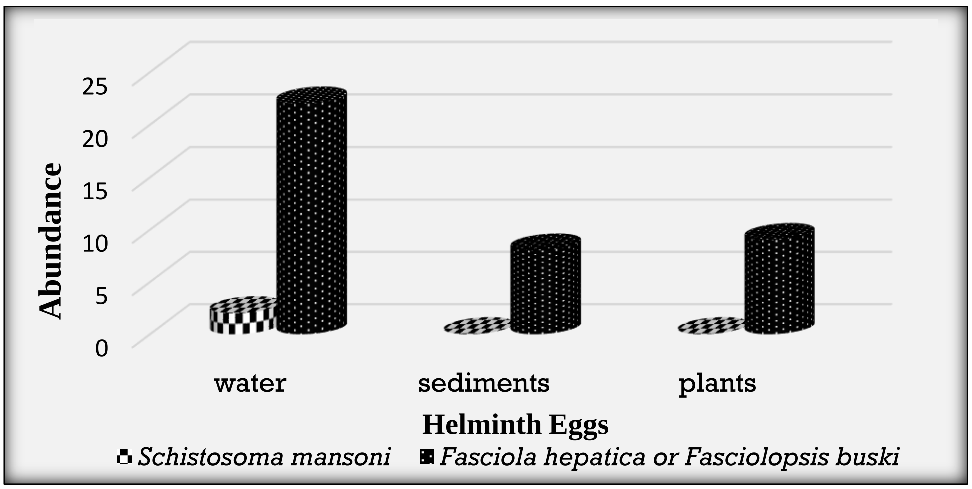

Figure 34: Trematode species abundance

iii. Cestode

As observed in Figure 38*Taenia Saginata, Taenia solium, Hymnelopis nana* of Cestodes were recognized. During performance evaluation of constructed wetland Motevali and his workers found *Hymenolepis nana* among the abundant nematodes (Motevalliet al., 2015).

Figure 35: ***Hymnelopis nana***

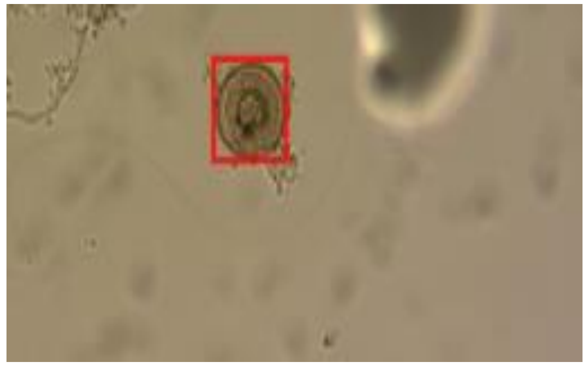

Figure 36: ***Hymnelopis dimunita***

Figure 37: ***Taenia Saginata***

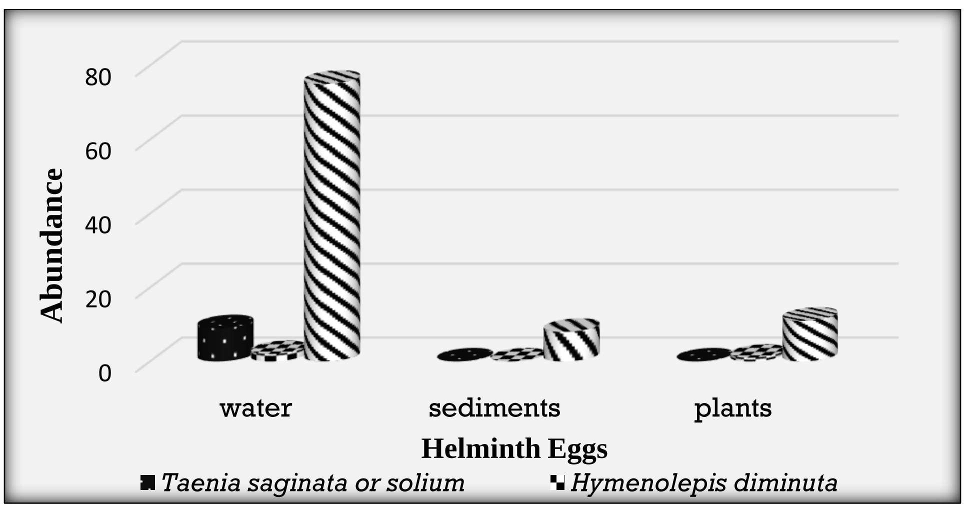

Figure 38: Cestodes species abundance

4.4. Presence of Heavy Metals in Wastewater, Sediments and Plants

According to EPA, heavy metals are regarded as primary pollutants and must be removed or minimized from water body that may or may not meet the environment (Virgen et al., 2018).

The mean concentrations of Nickel (Ni), Chromium (Cr), Cadmium (Cd), and Copper in water, sediments and plants are illustrated through boxplots/box and whisker plots. The line between the box shows the mean concentration of heavy metals while minimum (below bar) and maximum (upper bar) concentrations are given by the bars.

4.4.1. For Wastewater:

The concentrations of Cd, Cr, Cu and Ni fall into the ranges of 0.06-0.6115, 0.0001-0.0007 to, 0.02-0.1201 and 0.0025-0.0290mg/L respectively in this study. The minimum values in all heavy metals show that all values encountered the permissible limit of 0.1 mg/L for sewage wastewater (Pak-EPA, 2000). The maximum values for Cd and Cu were higher. Reason for Cd may be the use of household detergents, especially washing powders. Many authors, including Comber and Gunn (1996), have emphasized clothe washing cleaners and powders as major sources of Cd and Cu in domestic wastewaters (Laura et al., 2021). While Major source of Cu in wastewater is corrosion of household plumbing, faucets, and water fixtures.

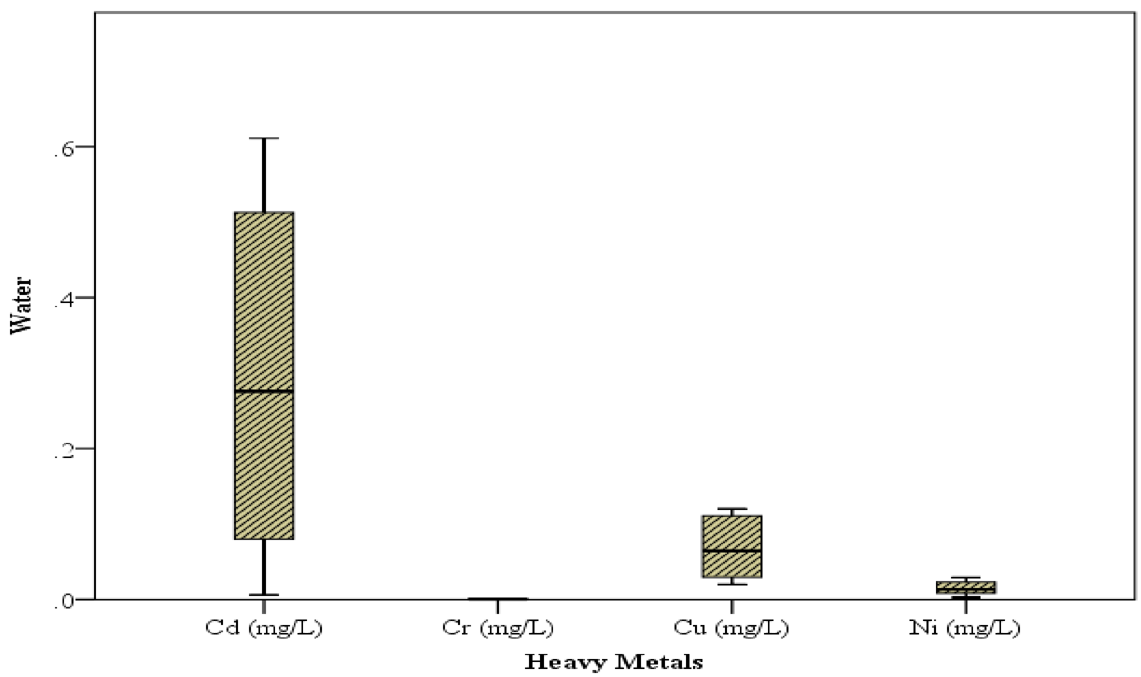

Figure 39: Heavy Metal Concentration in Wastewater

4.4.2. For Sediments:

In this study, the distribution of Cd, Cr, Cu and Ni in sediments were in the range from 0.615-1.321, 0.007-0.026, 0.04-0.320 and 0.027-0.032 mg/kg. These results are comparable with those previously reported by literature for Cd ranged in 0.6-1.9 mg/kg (Gill et al. 2017; Khan et al., 2009; Lesage et al., 2007), for Cr ranged from 0.04-1.3mg/kg (Kumari and Tripathi 2014; Lima et al., 2013; Lesage et al., 2007), for Cu ranged from 0.1-2.9 mg/kg (Rai et al. 2015; Pedescoll et al., 2015; Kanagy et al., 2008) and for Ni ranged from 0.09-2.9 mg/kg.(Sima et al., 2016; Terzakis et al., 2008; Lesage et al., 2007).

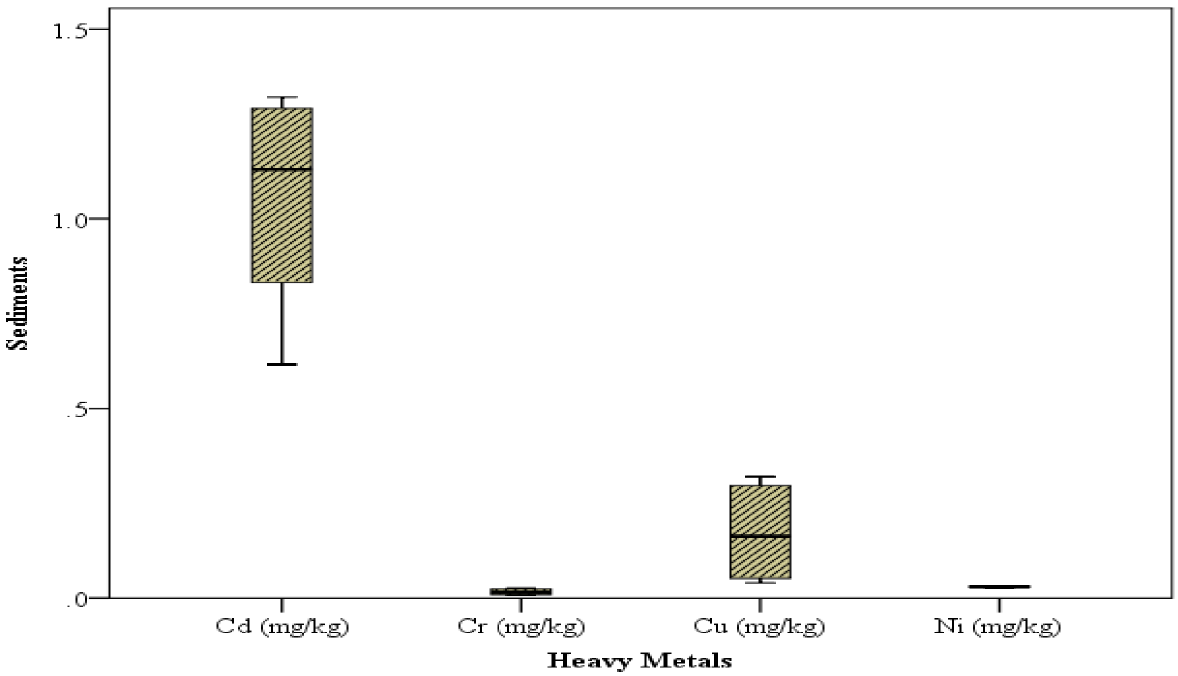

Figure 40: Heavy Metal Concentration in Sediments

4.4.3. For Plants:

In this study, the accumulation of Cd, Cr, Cu and Ni in plants were in the range from 0.601-1.319, 0.008-0.022, 0.048-0.319 and 0.038-0.02 mg/kg. These results are generally comparable with those previously reported by literature for Cd ranged from 0.9-5.2 mg/kg (Bonanno et al., 2018; Khan et al., 2009; Vymazal et al., 2007), for Cr ranged from 0.09-2.3mg/kg (Bonanno et al. 2018; Lima et al., 2013), for Cu ranged from 0.5 to 2.9 mg/kg (Pedescoll et al. 2015; Kropfelova et al. 2009; Jayaweera et al., 2008) and for Ni ranged from 0.45-5.1 mg/kg (Sricoth et al., 2018; Rai et al., 2016; Kumari and Tripathi, 2014).

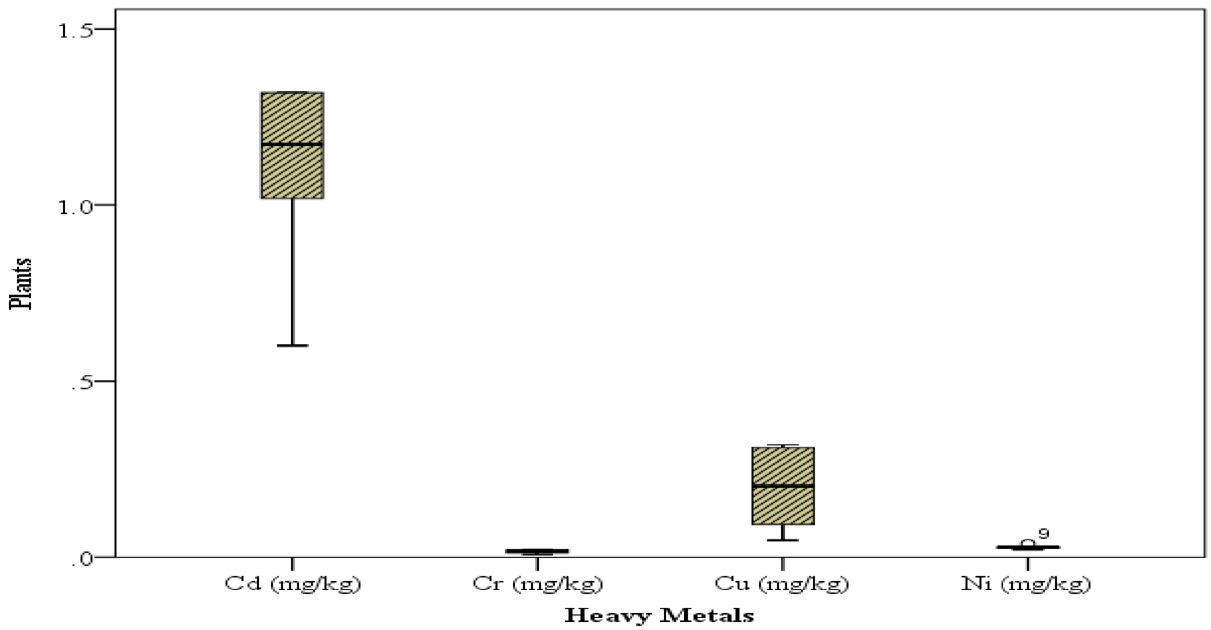

Figure 41: Heavy Metal Concentration in Plants

Chapter 5

5. Conclusions

Integrated Constructed Wetland systems (ICWs) is an efficient and cost-effective method for removing the contaminants from wastewater and reusing it for agricultural/horticultural purposes. An important issue that needs to be resolved is land limitation, however; the decision makers can regard and take benefit from the low energy needs and less capital costs. The results established from study deem it possible to state that from among all the nitrogen compounds whose removal efficiencies were compared, this factor was found the most unstable in winter as compared to autumn. Therefore, maintenance of ICWs by harvesting of plants is important before the wintertime to avert their decomposition in the ICWs and avoid them from acting as secondary pollutants.

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