HEAVY METAL REMOVAL FROM SYNTHETIC LEACHATE USING CONSTRUCTED WETLANDS

BY

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IN

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CERTIFICATE

This dissertation submitted by Ms Zahra Zaigham is accepted in its present form by the Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Sciences and Technology (NUST) Islamabad as satisfying the requirement for the degree of Masters of Environmental Science

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TO

MY FAMILY AND FRIENDS

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Zahra Zaigham

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LIST OF ABBREVIATIONS

MSW	Municipal Solid Waste
SWM	Solid Waste Management
VOCs	Volatile Organic Compounds
Fe	Iron
Pb	Lead
COD	Chemical Oxygen Demand
N	Nitrogen
P	Phosphorus
C	Carbon
S	Sulphur
CWs	Constructed Wetlands
HSSF	Horizontal Sub-surface Flow
VSSF	Vertical Sub-surface Flow
FWS	Free Water Surface
BOD	Biochemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TSS	Total Suspended Solids
DO	Dissolved Oxygen

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ABSTRACT

Municipal solid waste (MSW) leachate is characterized by high concentrations of chemical oxygen demand (COD) and metals which are adjudged to be hazardous for environment. A series of experiments was conducted for eight weeks to assess the removal efficiency of metals from synthetic leachate through 4 vertical subsurface flow poly cultured constructed wetlands. Four treatments (CW_1, CW_2, CW_3 and $CW_{control}$) were planted with *Pharagmites australis* and *Pharagmites karka* with varying contaminant concentrations in terms of nutrients and COD but fed with equal amounts of Iron and Lead . A control namely $CW_{Control}$ was also set which was provided with only Iron, Lead and COD of 10mg/L, 3mg/L and 1200mg/L respectively. It was observed that CW_3 which had highest amounts of nutrients (100mg/L of each Nitrogen and Phosphorus) and COD of 1600mg/L was clogged on 5th week. Removal trends for Pb were highest 30.4% in CW_2 which was fed with synthetic leachate containing 75mg/L each of Nitrogen, Phosphorus and 1200mg/L of COD. Iron removal was highest 20.9% in CW_2. Whereas Lead was more efficiently removed by constructed wetlands then Iron. Lead removal from CW_1 (fed with 50 mg/L each Nitrogen, Phosphorus and COD of 800mg/L) and CW_2 were high as compared to control which was not given additional nutrient supplements only heavy metals and COD of 1200mg/L. COD removal efficiency was also reasonable. An average COD removal of 42.7, 44.9, 18.9 and 37.3% for CW_1, CW_2, CW_3 and $CW_{Control}$ respectively was observed. Metal accumulation tendencies were also observed in selected wetland species at varying amounts of nutrients and COD. Roots of each specie accumulated more Pb as compared to shoots. Whereas Fe as a micro-nutrient was detected in roots and shoots of both plants in almost equal amounts. Over all about 1-2% of heavy metal removal was contributed by the plants. Two way ANOVA analysis ($P<0.05$) showed that there was no significant difference among plants in accumulating Pb and Fe against various treatments. Thus different concentrations of nutrients did not led to increase in metal accumulation within the plant tissues.

Chapter 1

INTRODUCTION

Increasing rate of urbanization and mechanization coupled with expanding population have evoked many environmental issues. One of the deleterious outcome of these activities is the production of high amounts of waste which created nuisance for the mankind and with this idea of specialized and efficient solid waste management is conceived. In today's world where population and development have swelled tremendously, effective waste management holds to be one of the most crucial social issue which must be dealt with in the context of sustainability and sound environment.

In developing countries open dumping is mostly preferred over systemized disposal due to several reasons, few of them are: low budget allocation for waste management, untrained man-power and unavailability of established technical assistance etc. (Ali et al., 2014). It is estimated that about 70-95% of disposal sites are open dumps in countries like Pakistan, Thailand and India (Visvanathan et al., 2003; Someya et al., 2010).

In Pakistan approximately 65,000 tons of municipal solid waste is generated per day out of which 51- 69% is collected by the municipal authorities and dumped into the designated disposal sites, whereas rest of the waste is left lying in vacant plots, streets, roadsides, railway lines, depressions, drains, storm drains , open sewers and depressions etc. which largely contributes to environmental degradation (Batool and Ch, 2009).Compaction of solid waste followed by sandwiching it between layers of soil and finally covering it with clay until dump capacity gets saturated is one of the common technique which improve the open dumpsite conditions. However many of the open dumps are left open which makes them hazardous towards the environment.

Landfill decomposition process involves many chemical and biochemical reactions which results in the generation of leachate. In open dumps this kind of situation is further triggered by the infiltrating water which usually comes through the rain. As the water infiltrates it draws in all the exchangeable and dissolvable components from the solid waste and transform into highly contaminated liquid, known as leachate (Kadlec and Zmarthie, 2010). In both cases i.e. landfill and open dumps leachate is considered to be highly contaminating towards environment as it holds a diverse range of contaminating, carcinogenic and xenobiotic chemicals including volatile organic compounds (VOCs), phenolic compounds, nutrients such as orthophosphates phosphorus, ammonia nitrogen, heavy metals (HMs) which pose a menacing threat towards environment and public health (Kietlińska and Renman, 2005; Oman and Junestedt, 2008; Aziz et al., 2010).

Depending upon its complex composition, leachate treatment technologies fall into two basic types, biological and physical/chemical. Systems on larger scale can be customized in terms of combining two or more treatment technologies depending upon the treatment goals (Raghab et al., 2013). Inanc et al., 2000 have reported a number of methods which have been adopted to treat the leachate which includes biological treatments using aerated lagoons, phytoremediation through wetlands, activated sludge and anaerobic lagoons. Physio-chemical treatments including pH adjustment, chemical precipitation, and coagulation by lime and potassium alum oxidation reduction have also been applied to leachate treatment.

In developed countries many physical-chemical wastewater treatment technologies are practiced to treat MSW leachate. Such approaches are technologically complex as well as financially expensive in nature. Furthermore, some of these treatment technologies require pre-treatment process as well (Renou et al., 2008). For a developing country like Pakistan all such factors rule out the possibility of these approaches to even exist. Keeping all these factors in mind.

Constructed wetlands can be considered as a viable option for the treatment of MSW leachate. These are artificially engineered systems based upon the principles of phytoremediation and are designed to mimic the functions of natural wetlands (Kröpfelová et al., 2009; Vymazal, 2009). It is regarded as low cost, easily operated and energy efficient technology (Langergraber, 2008). Similarly in leachate treatment using constructed wetland involves low capital investment, less maintenance cost, less energy and resource consumption and is simple to apply. Wetland plants can easily be grown in the vicinity or landfills or open dumps with almost negligible capital investment and can be run by relatively less skilled personals (Tuladhar et al., 2008).

1.1 Hypothesis

Since leachate is a high strength wastewater with considerable amounts of metals, nutrients and organic matter, its remediation is necessary before it is discharged. Constructed wetland can be considered as an eco-friendly approach to treat the effluent from waste materials. Hypothesis was to test whether nutrients enhance the metal uptake by the selected plants or not and their suitability in treating various concentrations of synthetic leachate.

1.2 Objectives

The objectives of the present study include:

1. Heavy metals removal by two selected wetland species from synthetic leachate having different chemical oxygen demand and nutrients concentrations.
2. Monitoring growth of both species in different concentrations of synthetic leachate using vertical subsurface flow system.

Chapter 2

LITERATURE REVIEW

Solid waste management is among the basic essential services provided by municipal authorities Disposal of solid waste within the sound context of environmentally hygienic and sustainability has always been an ultimate target for any country. Open dumping is usually practiced in developing countries threats which it poses towards environment are inevitable. One of the threats include generation of leachate which can wreak a havoc on surroundings if not treated or managed properly. Constructed wetlands offer an eco-friendly approach to not only add beauty to the dumping area but also treat wastewater emanating from there.

2.1 Solid waste management (SWM) practices in Pakistan

Pakistan has been ranked as 2nd largest country of South Asia region where as it holds the 6th rank in terms of population, it's been expected that it would further swell to 450 million people by 2050 (Gale and Fahey 2005). Pakistan's current practices of Solid Waste Management are not enough to cater the needs of its expanding population. In Pakistan approximately 55,000 tons of municipal solid waste is generated per day which makes the rate of waste generation per person to be 0.283 to 0.613kg/capita/day out of which 51- 69% is collected by the municipal authorities (Pak-EPA 2005). This waste usually includes household waste, construction debris, sanitation residue and even biomedical waste has also been witnessed at various dumpsites. Unfortunately none of the major cities of Pakistan owns an established Solid Waste Management system. Even Islamabad the planned Capital city of Pakistan does not possess a sound solid waste management plan or an engineered sanitary landfill. Open waste dumping are usually practiced to dispose MSW because of lack of resources and meager budget allotted to waste disposal services. These open dumps are a menace for the public health and ecosystems as it serve as source of pollution. Slack et al., 2005 stated that landfill emissions can be of many forms including gaseous release of Volatile Organic Compounds, airborne particulate matter and last but certainly not the least polluting leachate. Therefore these landfill/dumpsite management requires a check and balance approach.

Waheed et al., 2010 analyzed soil from the H-11 dumpsite Islamabad and reported serious contamination of Manganese, Copper and Antimony in dumpsite soil. Stress was laid upon the fact that this situation would get bad to worse if current disposal practices are continuously employed. In another study carried out by Munir et al., 2014 assessed the characteristics of various leachates collected from three various MSW open dumpsites i.e. Mehmood Boti, Saggian and Baggrian in Lahore. Out of which leachate from Baggrian dumpsite possess high amounts of various contaminants along with its pH being acidic. Leachate being highly contaminated wastewater can have inimical effects on the environment. According to Butt and Ghaffar, 2012 one of the worst environmental threats posed by an open dump is production of leachate and its percolation to the surrounding and groundwater bodies.

2.2 Generation of leachate

Topal,(2012) defined leachate as inherent water content of wastes that permeates through the heap of solid waste and draws out dissolved as well as suspended materials. Volume of leachate is further surged by external sources particularly by rainwater. The quantity and quality of leachate is directly related to the amount of water which passes through the waste and to the solubility and content of the discarded materials respectively (Esakku et al., 2007).The approximation of strength of leachate generated is crucial for evaluating possible surface and ground water contamination and it also indicates the degradation phase of the landfill (Frascari et al., 2004).

2.3 How leachate is harmful to environment?

Leachate can cause a serious contamination into the surrounding water resources and soil, even it can leach down to pollute ground water resources in case of landfill (Trebouet et al., 2001). It is marked as one of the toxic wastewaters due to the presence of heavy metal such as manganese, nickel, copper, zinc, chromium, ferric, cadmium and lead in considerable amounts which are absorbed by the leachate from various sources such as batteries, metal scrap materials, pharmaceuticals, fluorescent lamps etc. (Slack et al., 2005; Nordin and Amalina, 2006; Kadlec and Zmarthie, 2010). Kamarudzaman et al., 2011 reported concentrated amounts of nutrients in the form of PO_4-P and NH3-N in leachate samples collected from Malaysian Landfills. Leachate's untreated discharge can pose a serious threat towards water resources in the form of eutrophication which can lead to water basin satured with Nitrogen and Phosphorus substances as well ammonia (Hassidou et al., 2010). The contamination potential of leachate can be assessed by Leachate Pollution Index (LPI) its value ranges from 5-100, it expresses the potential of contamination from a landfill/dumpsite based on monitoring of 18 parameters of leachate. LPI higher than 7.5 indicates polluting leachate (Kumar and Alappat, 2005).Municipal Solid Waste (MSW) leachate can impart several detrimental impacts on aquatic and terrestrial ecosystems (Aziz et al., 2004).Hence the treatment of leachate before its discharge into receiving water is required in order to curtail its dangerous effects.

2.4 Potential contaminants in leachate

Characterization of leachate not only reflects the composition of solid materials but also indicates the biodegradation phase through which a landfill/dumpsite is being passed through (Renou et al., 2008). Chemical composition of leachate change with the passage of time, and it follows a general trend of reduction of leachate contamination index with the reduction of site age. (Chian *et al*., 1977). Munir et al. 2014 carried out a study on three open dumpsites located at

Lahore having different operational ages. Highest Leachate Pollution Index of the dumpsite was observed for the sites which were less than 5 year old this supported the fact that leachate resulting from a young landfill/dumpsite is more toxic and unstable as compared to the mature leachate. But that does not rule out the toxicity associated with the mature leachate.

Although leachate emanating from old landfills are stable but they contain high amounts of ammonium nitrogen and other persistant natured compounds (Kulikowska, 2012). According to Wiszniowski et al., 2006 despite of prevailing concentration changes which are directly or indirectly related to an amalgamation of interrelated factors, the composition of landfill/ dumpsite leachate can be categorized on the basis of four crucial categories of contaminants which are as follows:

2.4.1 Dissolved organic matter

Leachates contain organic matter which are composed of molecules of varied organic origin and compositions, it is measured in terms of Chemical Oxgen Demand and Biological Oxygen Demand (Lee and Nikraz, 2014). They are contemplated as one of the most problematic parameter associated with the MSW landfill/dumpsite leachate treatment. Leachates are often characterized by high values of Total Organic Carbon (TOC), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS) and Biological Oxygen Demand (BOD) (Słomczyńska and Słomczyński, 2004). Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD_5) are used to evaluate organic content in MSW leachate. According to Kurniawan *et al*., 2006 BOD_5/COD ratio gives a fair evaluation of the maturation stage of landfill/dumpsite leachate i.e. for a young leachate the ration tends to hold value between 0.4-0.5.

High levels of organic contaminants in leachate result into hindered diffusion of Oxygen thus leading to low Dissolved Oxygen (DO) which makes leachate waters toxic towards aquatic organisms (Johansen and Carlson, 1976). According to Englehardt *et al* ., 2006 young leachates may contain 36 times higher Chemical Oxygen Demand (COD) as compared to untreated sewage where on the other hand old leachate may contain COD corresponding to untreated sewage but it holds many other refractory high molecular-weight organic contaminants then sewage. Mature leachate is considered as stable yet its treatment is challenging because of the less biodegradable organic fraction thus leading to be a potential source of toxicity to the environment (Deng and Englehardt, 2008).

2.4.2 Metals

Elements having density greater than 5g/cm^3 are characterized as ‘Heavy Metals’, these are stable metalloids which include Nickel, Molybdenum, Cadmium, Zinc, Chromium, Iron, Titanium, Lead, Cobalt, Mercury etc. (Nies,1999). These elements are naturally present in earth’s crust in minute quantities but as a consequence of accelerating anthropogenic activities such as mining, urban sewage, landfill leachate, smelters, tanneries, textile and chemical industry etc. their amount has been increased tremendously, which has taken its toll on the environment (Khan et al., 2009). These metals make their way into hydrosphere as well as lithosphere from where they eventually pollute food chain. Heavy metals pose serious threats towards environment and human health because of their toxicity and refractory nature (Olatunji et al., 2009). Waste that constitutes discarded electronic appliances, electro-plated objects, worn out batteries etc. contribute towards heavy metals in dumpsites (Kanmani and Gandhimathi, 2013). Kanmani and Gandhimathi 2013 explains that as the degradation of waste proceeds it trends to produce leachate under acidic conditions which leads to high metal absorption in leachate.

Metals such as Iron (Fe), Copper (Cu), Chromium (Cr), Nickel (Ni), Lead (Pb) and Mercury (Hg) are bound to be present in it. A close environmental surveillance is required to evaluate heavy metal concentrations in leachate in order to curb the groundwater contamination, it will also help in suggesting appropriate remedial measures (Esakku et al., 2003). Following table describes the lethal impacts of various heavy metals on human health:

Table 2.1 Adverse impacts of heavy metals on human health

Heavy metals	Effects on Human Health	Permissible limit (mg/L)	References
Arsenic (Ar)	Bronchitis, Dermatitis, Poisoning	0.02	Mandal and Suzuki, 2002; Bisson and Frimmel, 2003
Cadmium (Cd)	Renal dysfunction, gastro-Intestinal and reproductive disorders, Cancer	0.06	Mortada et al.,2004
Lead (Pb)	Effects on Reproductive and Central Nervous System, acute or chronic damage to nervous system leading to developmental disorders	0.1	Naseem and Tahir, 2001
Manganese (Mn)	Causes Neurotoxicity, Tremors, Gingivitis, Minor behavioral changes	0.26	Mergler, 1999; Levy et al., 2003
Chromium (Cr)	Adverse impacts on Nervous system, fatigue	0.05	Liao et al., 2011; Were et al., 2014
Copper (Cu)	Hematic as well as hepatic damage, gastrointestinal problems	0.1	Pizarro et al., 1999; Georgopoulos et al., 2001
Zinc (Zn)	Damaging towards Respiratory and nervous system.	15	Plum et al., 2010

2.4.3 Excess nutrients

Landfill/dumpsite leachate is usually characterized by excessive nutrients which are present in the form of phosphates, nitrates and ammonia. According to literature review raw leachate from landfills can have ammonical nitrogen (NH_3-N) as high as 2,000-5,000mg/L. Decomposition of proteins and amino acids results in the ammonium nitrogen formation, it is not only considered as long-lived pollutant but can also lead to serious adverse impacts towards aquatic organisms on exposure (Kurniawan et al., 2006). On the other hand a substantial portion of MSW leachate is contributed by phosphates(PO_4^{-3})and nitrates (NO_3^-). Degradation of organic waste constituting phospho-proteins and phospholipids contributes towards production of phosphates in leachate (D’Souza and Somashekar, 2013).

It is speculated that combustion processes at dumpsites lead to the release of N_2O which contribute towards high nitrate values in leachate (Ojoawo et al., 2012). Heavy nutrient loading in leachate depicts that it as a potential source of eutrophication in water bodies which can adversely impact aquatic life by depleting dissolved oxygen (Malik et al., 2013). Where some also view leachate as a rich source of nutrients for fertilizing purposes but due to presence of hazardous contaminants and higher salinity level, its application over food crops is hesitant (Mor et al., 2013).

2.4.4 Xenobiotic compounds

Xenobiotic compounds are those compounds which are not naturally produced they are produced due to anthropogenic activities. They are normally referred as recalcitrant in nature because of their ability to withstand any degradation (Connell, 1989). Sources of XOCs in MSW leachate include laundry detergents, toothpastes, insecticides, baby toys etc (Gordon et al., 2008; Capdevielle et al., 2008). However the type and the enormity of the xenobiotic compounds vary from source to source but even their minor release is of great concern because of their persistent nature. The most commonly and abundantly found XOCs are the aromatic hydrocarbons which includes phenols, benzene, toluene, ethylbenzene etc and as well as chlorinated hydrocarbons such as tetracholoroethylene and tricholoroethylene (Christensen et al., 2001). Another major XOC identified in this category is herbicide Mecoprop or Methylchlorophenoxypropionic acid (MCPP) (Oman and Hynning, 1993).

With the passage of time the concentration of XOCs are anticipated to deplete which is conditional on the degradation phase of the landfill i.e. aerobic and anaerobic and the volatilization of the compounds in landfill gas (Kjeldsen and Christensen 2001). Nevertheless, evaluating XOCs in landfill leachate is difficult due to lack of understanding of their behavior, high cost testing and sampling protocols (Malik et al., 2013).

Hence all the potential contaminants present in MSW landfill leachate makes the area around it prone to various threats. This calls for an efficient management of leachate. An inappropriate management of MSW leachate results into sanitary and epidemiological hazard. Table 2.4 explains the various eco toxicological effects posed by various contaminants present in the leachate (Tchobanoglous, 2009).

Table 2.2: Typical information on various contaminants present in MSW leachate

Contaminants in MSW Leachate	Typical Range in Leachate (mg/L)	Eco toxicological effects	NEQ's For Wastewater effluents (mg/L)
Chemical Oxygen Demand (COD)	800-60,000	High amounts deoxygenates surface water disrupting aquatic survival	150
Biochemical Oxygen Demand (BOD_5)	500–30,000	Reducing DO levels	80
Total Suspended Solids (TSS)	200-2,000	Smothering aquatic life, adsorbed pollutants lead to toxicity	150
Ammonia Nitrogen	10-800	Eutrophication, Soil acidification, dangerous to inhalation	40
Total Phosphorus	5-100	Eutrophication creates hypoxic environment in the water bodies	-
pH	4.5–7.5	High or low pH can both disrupt natural ecological processes	6-10
Total Hardness	300–10,000	Increases salinity leading to alteration of an ecological system	-
Total Iron	50-1200	High doses disrupts hormones	
Alkalinity	1,000-10,000	Alter the water quality thus disrupting ecological balance	-
Dissolved Xenobiotics -Phenols -Benzene -Naphthalene -Tetrachloro-ethylene -Toluene	1-1200* 1-1630* 0.1-260* 0.1-250* 1-12300*	Highly toxic, Biomagnification and bioaccumulation leading to human toxicity	0.1 (Phenols)
Magnesium	50-1500	Respiratory problems, nausea, cardiovascular problems, Mortality of tadpoles, Ceriodaphnia dubia, rainbow trout has been reported	-
Sulphates	50-1000	Causes diarrhea	600

2.5 Wetlands

Wetlands also known as marshes are those areas which are always inundated with stagnant or slowly flowing water. They cradle a rich biodiversity of plants and animals. Equipped with variety of aspects which are renowned as of significant importance from ecological, economic and social point of view. They exhibit a unique role of acting as earth’s kidneys by filtering pollutants from water as it passes through lakes, streams and oceans. Following this concept, scientists and engineers from all over the world have successfully developed the replicas of natural wetland systems known as Constructed or Artificial Wetlands. According to Lambert, 2003 constructed wetlands are designed to filter wastewater using natural processes found in natural wetland ecosystems mainly run by vegetation, soil and microorganisms related to wetland biota.

2.6 Constructed wetlands

Constructed wetlands were the brain child of a German Scientist named Käthe Seidel who installed an artificial wetland in the premises of Max Plank Institute Plön. After the success of these man-made wetlands they were adopted across the Europe and United States of America. Several processes such as sedimentation, adsorption, complexation, uptaking by plants, and microbial assisted reactions including oxidation and reduction are involved in the working mechanism of constructed wetlands (DeBusk,1999).

Constructed wetlands being a biogeochemical system with sun as a main source of energy have countless advantages over other modern technologies such as it is low on budget , requires minimal maintenance , provides high treatment efficiency, beautifies the surroundings and is an environment friendly technology which can be maintained by relatively less skilled personals. For all the reasons outlined above this green technology has widely been accepted by countries like America, India , Srilanka , Portugal, Greece, Belgium, Spain, Mexico and Italy (Lesage et al., 2007; Chavan et al., 2012).

Over the last couple of decades wetlands are been used in treating various kinds of wastewater such as dairy wastewaters, phenol wastewaters, domestic wastewaters, pharmaceutical wastewaters, industrial wastewaters and municipal landfill leachate (Vymazal, 2005 ; Vymazal and Krőpfelová, 2005; Khan et al., 2009; Kadlec and Zmarthie, 2010**;** Bhatia and Goyal, 2014).

2.7 Types of constructed wetlands

Fundamentally, there are two types of constructed wetlands depending upon their water levels these various types may be integrated into one another in an on-site system for any kind of wastewater treatment (Kadlec and Wallace, 2008). These two basic types include:

a. Free water surface Constructed Wetlan b. Subsurface flow Constructed Wetland

2.7.1 Free water surface constructed wetland

Free water surface simply known as surface flow constructed wetlands involve open shallow ponds or channels with a water depth of 20-40cm long and 20-30cm of anchoring soil, these are densely populated with floating as well as emergent vegetation. It’s one of those types of CWs which is closely related to the natural wetlands for this reason a variety of wildlife is attracted to these artificially constructed wetlands. Figure 2.1 depicts FWS-Constructed Wetlands with emergent macrophytes:

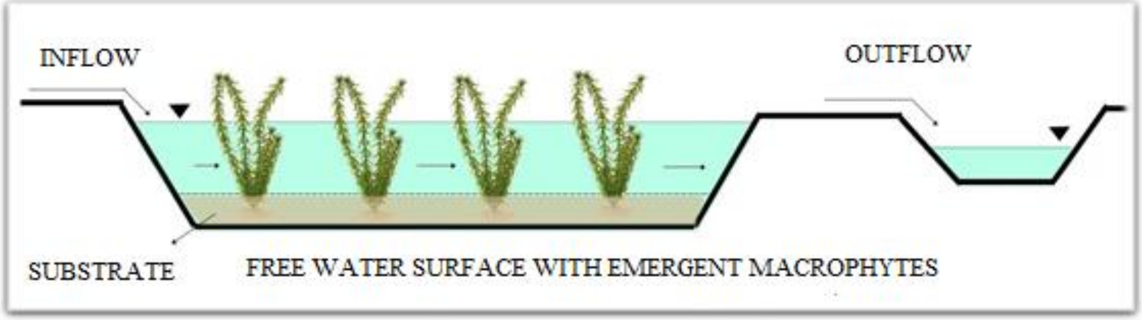


Figure 2.1 FWS-constructed wetlands with emergent macrophytes

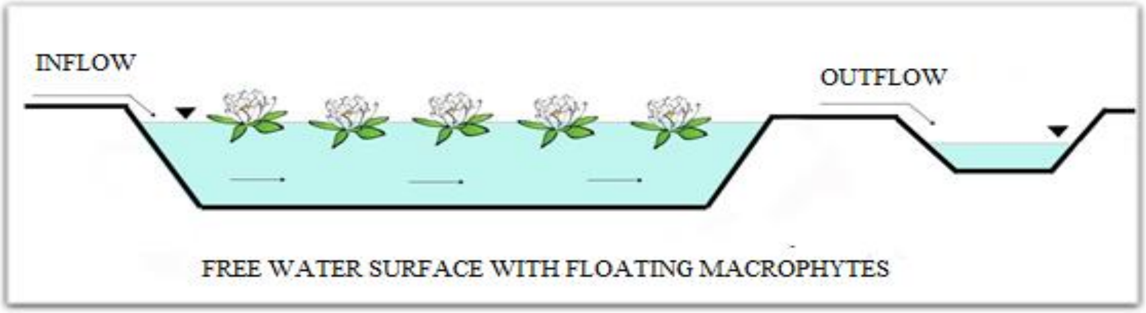


Figure 2.2 FWS-constructed wetlands with floating macrophytes

In FWS water flow and infiltration can be controlled through liners, berms or dikes. Livestock wastewater, Agricultural wastewater ,Urban and Industrial wastewaters are among the few examples of wastewaters which can be treated through FWS CWs because they can withstand water level and flow fluctuations (Vymazal, 2010).Other than that FWS CWs have proven to be effective in removing organic matter through microbial activities and settling of colloidal particles. Thick cover of macrophytes play an efficient role in the settlement as well as filtration of suspended solids (Kadlec and Wallace, 2008).

Nitrogen is removed primarily through nitrification (in water column) and subsequent denitrification (in the litter layer), and ammonia volatilization under higher pH values caused by algal photosynthesis. Phosphorus removal efficiency is usually low because of limited contact of water with soil particles which adsorb and/or precipitate phosphorus. Plant uptake is as temporary storage because the nutrients are released to water when the plant decay. However they have been applied in the treatment of various kinds of wastewaters such as wood waste leachate, refinery process effluent, livestock wastewaters, agricultural runoff etc. (Masbough et al., 2005; Tanner et al., 2005; Vymazal, 2010).

2.7.2 Subsurface flow constructed wetlands

The flow in subsurface wetlands occurs under the surface and within the soil planted with emergent aquatic plant species. Such engineered wetlands are synonymous with terms such as

‘Rock-Reed filter’ and ‘Root-Zone Method’. As the water flows beneath the surface of soil this reduces the risk of mosquito breeding this feature gives an edge to this type constructed wetlands over FWS where there the water is exposed to surface. Subsurface flow constructed wetlands are further divided into following types:

a. Horizontal Subsurface Flow Constructed Wetlands

b. Vertical Subsurface Flow Constructed Wetlands

c. Hybrid Constructed Wetlands

2.7.2.1 Horizontal subsurface flow constructed wetlands

The design of HSSF constructed wetlands consists of a rectangular reed bed which consists of soil, sand and gravel with an average depth of 0.6 to 0.8m. A slight inclination difference of about 1-3% is given between the inlet and outlet points in order to ensure complete drainage of wastewater from the system. Figure 2.3 shows the diagrammatic representation of HSSF-Constructed Wetland

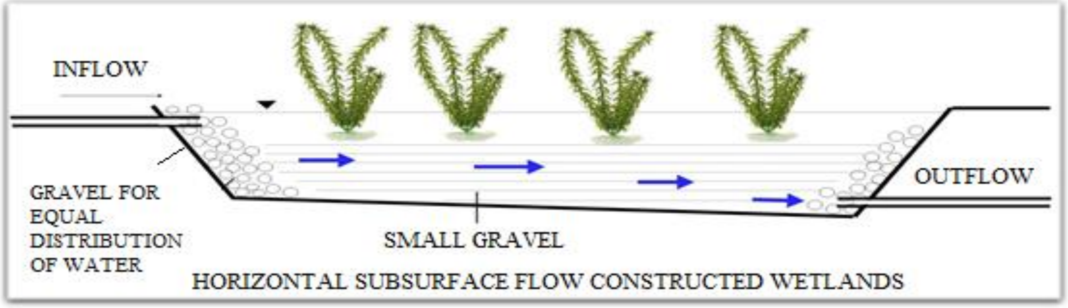


Fig 2.3 Horizontal subsurface flow constructed wetlands

In HSSF constructed wetlands water is more or less moving parallel to the surface. Soil acts as a substrate for plants to grown on. According to Cooper et al., 1989 the wastewater flows under the soil surface where it contacts with various zones which are differentiated on the basis of availability of Oxygen i.e. aerobic, anoxic through the plants’ roots and filtration medium. The aerobic zones lies around the roots and rhizomes of the wetland vegetation that leak oxygen into the substrate. During the passage of wastewater through the rhizosphere, the wastewater is treated by microbial activities. HF wetland can effectively remove the organic pollutants (TSS, BOD_5 and COD) from the wastewater. But due to the limited transfer of oxygen inside the wetland and low sorption capacities of filter mediums like gravel, sand, crushed pebbles etc. the removal of ammonia and phosphorus is limited respectively (Vymazal, 2005). However such conditions can be improved through aeration and by using high sorption capacities substrates. Drizo et al., 1999 carried out a study in United Kingdom. According to which shale and fly ash were reported as the best substrates in terms of removing phosphates in CW as these two materials have high P adsorption capacities.

Rousseau et al., (2004) mentioned HSSF CW suitable for aerobic post-treatment of wastewater. This type has widely been applied for treatment of various kinds of wastewaters, 60-90% removal of organic loading and suspended solids was found in contrast to nutrients N and K with removal rates of 50-60% (Mantovi et al., 2003; Schulz et al., 2003; Shuib et al., 2011).

2.7.2.2 Vertical subsurface flow constructed wetlands

FWS and HF constructed wetlands cover large foot print, in order to reduce it the concept of Vertical Flow constructed wetlands was introduced. Because of their packed spatial appearance, they are also known as Compact Vertical Flow Reed Beds. They were also said to be originally introduced to ventilate anaerobic septic tank effluents by Seidel (Weedon, 2001). Nonetheless VF CWs were not readily accepted due to their maintenance and high operational requirements.

In VSSF constructed wetlands which are also based on soil, sand and/or gravel substrate, influent usually in large volumes is inserted vertically throughout the surface from where it infiltrates and is drained through the substrate via network of drainage pipes. The next dose of influent is provided when the water is completely drained out of the VF units so that the air could refill the bed (Vymazal, 2011). Figure 2.4 shows Vertical subsurface flow Constructed Wetlands

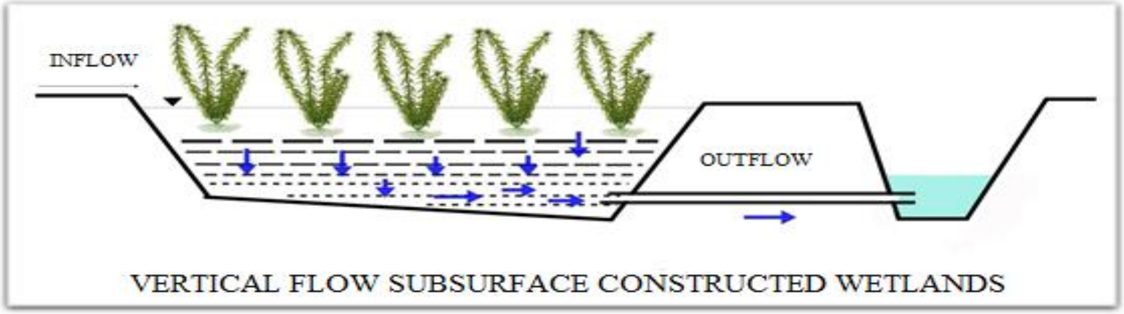


Fig 2.4: Vertical subsurface flow constructed wetlands

In general vertical subsurface flow constructed wetlands are considered more efficient than any other artificial wetland because of the aerobic conditions which usually exist in these kinds of constructed wetlands. Morari and Giardini, 2009 reported removal efficiencies of COD,BOD,N and K in VSSF CWs which were greater than 86%. Such kinds of CWs shows high nitrification rate because of the aeration capacities which they possess (Abou-Elela et al., 2013; Platzer,1999).Kamarudzaman et al., 2011 reported 91.5-92.2% and 94.7-99.8% removal of Fe and Mg respectively through VSSF constructed wetlands.

2.7.2.3 Hybrid constructed wetlands

Each type of constructed wetland has one or two limitations associated with itself in terms of removal efficiency, these limitations are different to each type. For instance denitrifying ability of Vertical flow System is minimal because of the aerobic conditions whereas on the other hand Horizontal flow Constructed Wetlands show sterling capabilities in denitrification this is where the idea of Combined Systems/Hybrid Constructed wetlands also known to be as ‘Fourth Generation of Constructed Wetlands’ originated (Vymazal, 2005). This system combines various types of constructed wetlands (usually HF and VF are combined) to attain more effective removal efficiencies of various contaminants. Masi et al., 2002 found the removal of total Nitrogen TN to be 50-90% where as total phosphorus was around 20-60% from winery wastewater through

Hybrid Constructed Wetlands. Figure 2.5 presents schematic diagram of Hybrid constructed wetlands

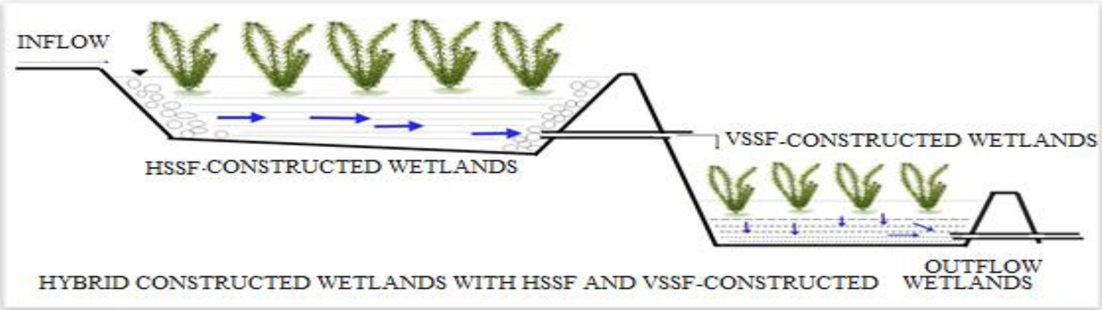


Figure 2.5: Hybrid CWs integrated with HSSF-CW and VSSF-CW

2.8 Components of constructed wetlands

A typical constructed wetland consists of three major constitutes which includes vegetation, substrate and micro-organisms.

2.8.1 Vegetation

Macrophytes hold a very unique position in the tapestry of biodiversity. Scientists estimate that there are millions or more plant species which thrive in wetlands (Alonso, 2008). Macrophytes also termed as 'Aquatic Plants' not only sequester contaminants directly into their tissues but also provide sites for microbial activities particularly around the rhizosphere which escalates a variety of chemical and bio-chemical reactions that enhance the removal efficiency of various pollutants in constructed wetlands (Jenssen et al., 1993). They also render their services physically by controlling erosion and insulating the reed bed surface during winters later one is considered an important function in HF and VF constructed wetlands especially for the countries which lie in temperate and cold regions (Brix, 1997; Mander and Jenssen, 2003).

Hence it becomes evident that plants used in constructed wetlands must have high tolerance towards nutrient and organic rich loadings and should possess ample amounts of below ground biomass (roots and rhizomes) which normally they do possess. Wetland plants can chiefly be categorized in three types as shown in the Figure 2.6

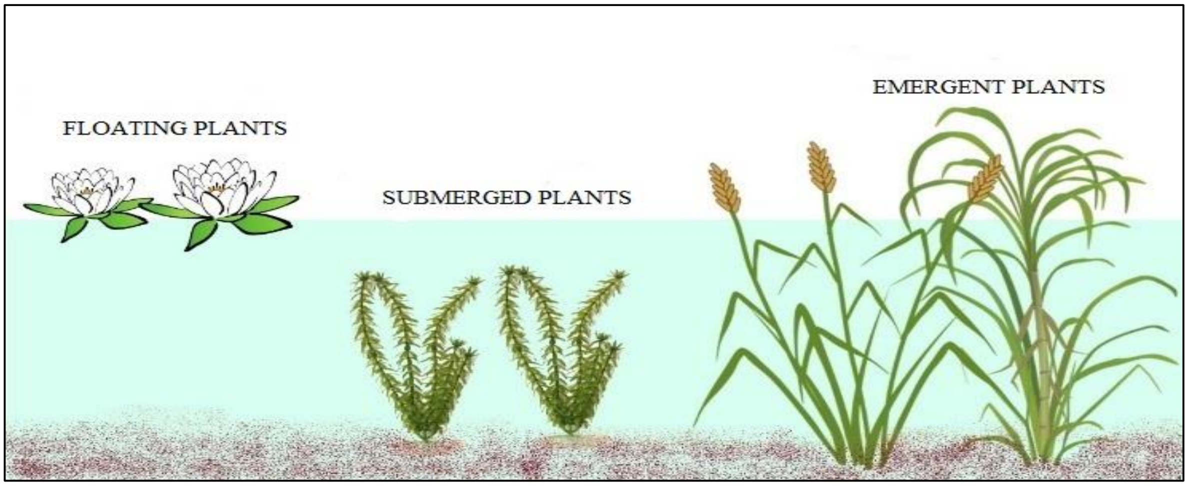


Figure 2.6: Types of wetland plant species

Floating plants as the name suggests are free floating plants, they are not anchored to substrate. Lemnaceae is one of the known worldwide family of free floating plants which comprises of duckweed, Wolffiella and Wolffia (water meal). Large species including *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce) which belong to family Pontederiaceae and Araceae respectively are usually used as free floating macrophytes in free water surface constructed wetlands (Cronk and Fennessy, 2001). Water hyacinth and water lettuce have wide fibrous branching roots that are suspended down into the water column. This extensive root system play a vital role in absorption of various contaminants.

Emergent species are those which are embedded in the substrate but emerge the water body surface. Such species have a high potential of treating wastewater since they provide maximum microbial interactions by serving as a microbial habitat. Examples of emergent plant species includes *Scirpus longii* (bulrush), *Typha latifolia* (cattail) and *Phragmites communis* (reeds) (Kadlec, 1999).Aslam and co workers assessed HM removal efficiency from refinery wastewater through typhae lattifolia planted constructed wetlands. Removal efficiency improved gradually making removal of Fe, Cu and Zn to be 49%, 53% & 59% respectively.

Through experimentation it has been observed that floating species retain large amounts of contaminants in their tissues as compared to emergent species because of the fact that they are directly in contact with the wastewater unlike emergent plants which are embedded in soil for that it retains less proportions of contaminants as compared to floating plants (Maine et al., 2009).

The third category is of submerged plants as the name speaks for itself these plants are completely submerged into water their bottom is either attached to the substrate or free floating. In submerged species, all photosynthetic tissues are normally underwater. *Hydrilla verticillata* (hydrilla) and *Ceratophyllum demursum* (coontail) are among the few examples of submerged plants.

There are several known species which have proven to be efficient in terms of accumulating pollutants from the water. In 2008 Kumar *et al*., studied various species including *Ipomoea aquatica* Forsk, *Eichhornia crassipes*, (Mart.) Solms, *Typha angustata* Bory and Chaub, *Echinochloa colonum* (L.) Link, *Hydrilla verticillata* (L.f.) Royle, *Nelumbo nucifera* Gaerth. and *Vallisneria spiralis L* for phytoremediation of heavy metal contaminated water, out of which *Typha angustata* and *Ipomoea aquatic* were said to be the most potential candidates in terms of sequestering large amounts of heavy metals.

Kamarudzaman et al., 2011 compared the efficiency of *Limnocharis flava* planted horizontal and vertical subsurface flow constructed wetlands in removing Fe and Mn from the landfill leachate. Studies revealed that the highest amount of heavy metals were present in the roots for both the systems ie HSSF and VSSF constructed wetlands 0.728 mg/g (VSSF) and 1.117 mg/g (HSSF) for Fe and 0.223 mg/g (VSSF) and 0.362 mg/g (HSSF) for Mn, respectively . Rai et al., 2013 reported 84%, 76% and 86% removal of NO3-N, PO4-P and NH4-N through onsite poly-culture (*Typha latifolia, Phragmites australis, Colocasia esculenta, Polygonum hydropiper, Alternanthera sessilis and Pistia stratoites*) constructed wetlands treatment of sewage. According to Baldantoni et al., 2004 *Phragmites communis* has the potential of accumulating more macronutrients (C,N,P,S) in their leaves as compared to their roots and stems. *Eichhornia crassipes, Phragmities australis, Phragmities karka, Typha latifolia Hydrilla verticillata* and *Salvinia* have also been subjected to remove pollutants from various kinds of wastewater and they have showed reductions in the selected pollutants (Du Laing et al., 2003; Tiwari et al., 2007; Dhote and Dixit 2009).

Some scientists argue that macrophytes provide temporal storage towards pollutants because upon senescence of plants the sequestered pollutant are released back into the surrounding, which contaminates the site once again. While some scientists suggest to incinerate the contaminant laden plants. Following table shows the efficiencies of various macrophytes in terms of removing various contaminants from wastewater:

Table 2.1 Contaminant removal efficiencies by constructed wetlands planted with different aquatic plants

Specie used	Common Name	Wastewater Treated	Removal efficiencies	References
Phragmites australis (Cav.) Trin	Common Reeds	Municipal and animal farming wastewater	93.5%,94.8% ,25%,20% for COD, BOD, TKN, NH_3 respectively	Abou-Elela et al., 2012
Typha latifolia	Common cattails	Refinery Wastewater	49%,53% and 59% for Fe, Cu and Zn	Aslam et al., 2010
Hydrilla verticillata	Hydrilla	Pb contaminated wastewater	98% removal of Pb after one week exposure	Maria et al., 1999
Typha domingensis Pers	Southern Cattail	Industrial effluent	30%, 13%,41%,38% and 23% for Cr, Ni, Zn, P and N	Maine et al., 2009
Eichhornia crassipes (Mart.) Solms	Common water hyacinth	Industrial wastewater	53%, 64%, 65%, 47%, 94% and 30% for TSS, BOD,DO, nitrate-nitrogen,cadmium and iron respectively.	Ajayi and Ogunbayio, 2012
Phragmites karka Retz.	Tall reeds	Domestic wastewater	100% for organic nitrogen,98.7% Coliforms,88.4% Turbidity,79% TSS,77.8% COD, 65.7% for BOD	Vipat et al., 2008

2.8.2 Substrate

In constructed wetlands substrate is considered to be the plinth of the system where all the biotic and abiotic components interact. Substrate which may include soil, sand, gravel, rocks and organic materials, is important because it sustains diverse range of life forms in natural/artificial wetlands, acts as medium which experiences many of the chemical reactions taking place in the system and also retain numerous contaminants in itself through the process of adsorption (Stottmeister et al., 2003).Substrates which have high adsorption capacities along with high ecological activity are preferred in constructed wetlands.

2.8.3 Micro-organisms

One of the important component of Constructed wetland is the diversity of microbes which it harbors. Micro-organisms play a vital role in altering and mineralizing nutrients and organic pollutants into simpler and biologically useful forms. Macrophytes have immense above and below ground biomass, the latter one provides a large surface area for growth of microbial bio-films (Brix, 1997). In subsurface flow constructed wetlands, aerobic processes such as nitrification only dominate around roots and on the rhizoplane whereas areas which are deprived of oxygen i.e. anoxic areas have anaerobic microbes which facilitates processes including denitrification, sulfate reduction or methanogenesis etc. (Stottmeister et al., 2003; Kayser and Kunst, 2005).

2.9 Working Mechanism behind Constructed Wetlands

The working mechanism behind Constructed wetlands is a combination of several physical, chemical and biological processes which are simultaneously involved in transformation and removal of pollutants from the influent. Figure 2.7 shows a diagramatic view of removal processes in Constructed wetlands

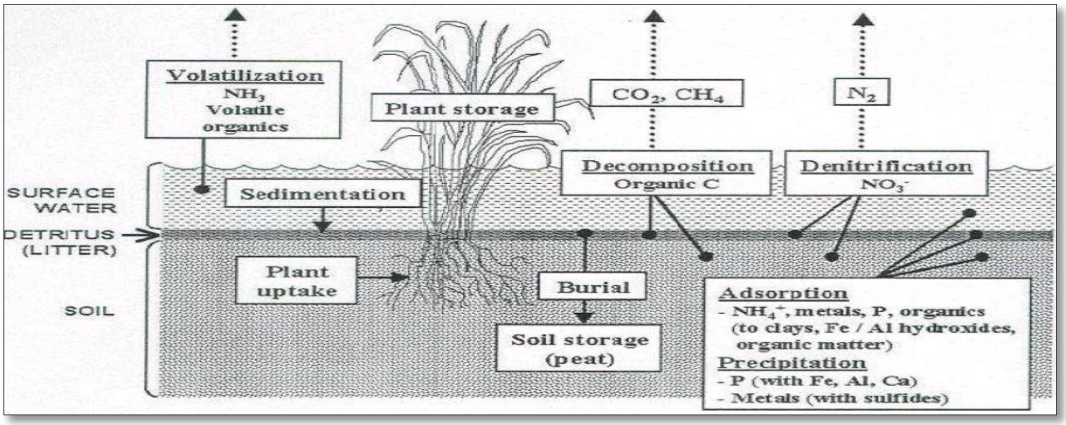


Fig 2.7: Removal Processes Occurring in Constructed Wetlands (DeBusk, 1999)

2.9.1 Physical Processes

Constructed wetlands tend to provide high efficiency to remove suspended matter present in the influent through physical processes including sedimentation, filtration and adsorption. Vegetation plays a key role in carrying out physical processes in the case of subsurface flow CWs this role is shared by substrate as well (Korboulewsky et al.,2012). Aquatic macrophytes not only helps in trapping sediments through their root structure but also pose resistance towards water that flows through the wetlands thus induce a laminar flow to the water. Norton, 2014 explained that as water flow gets decelerated by the vegetation the process of sedimentation of suspended materials gets enhanced. Contrary substrate acts as filter bed which allows the process of filtration allowing only selective materials to pass through some of them also get adsorbed to the substrate surface such as phosphorus (Comeau et al., 2001).

2.9.2 Biological Processes

Constructed wetlands involve an array of biotic processes which are involved in the removal of various pollutants. Plant uptake and microbial activities form the core of such processes. In wastewaters many forms of essential plant nutrients including nitrates, phosphates, ammonium and sulphates etc. are present as contaminants, these are readily taken up by the plants and are sequestered within their tissues. Macrophytes also have this inherent capability to store toxic elements within their body such as Cadmium, iron, lead etc. (Dordio et al., 2008). Allen, 1997 studied the mutualistic relationship between aquatic plants and microbes, he observed that aerial stems of macrophytes by virtue of large internal air spaces transport O_2 to the rhizosphere which enables aerobic microbes to decompose the complex compounds into simpler ones. The rhizosphere of macrophyte plant is said to be the hyperactive zone in terms of microbial presence and activities. Micro biota plays a major role in sequestering Phosphorus (P), but its sequestration rate depends upon the trophic level of the water body i.e. in less enriched sites microbial storage of phosphorus is more as compared to the eutrophic sites. In Constructed wetlands microbial metabolism is also responsible for the removal of inorganic nitrogen (nitrate and ammonium), which ultimately leads to the production of N_2 gas which is eventually lost to the atmosphere. These coupled processes of nitrification and denitrification are of high importance in terms of cycling and bioavailability of Nitrogen in wetlands. The coupled processes of nitrification and denitrification are universally important in the cycling and bioavailability of nitrogen in wetland (Vymazal, 2007).

2.9.3 Chemical Processes

In addition to biological and physical processes, chemical processes also contribute in making constructed wetlands an efficient eco-friendly technology. The two dominating chemical processes occurring in wetlands include adsorption and precipitation. Pollutants which are oxidized through redox reactions, photolysis, ionic exchange hydrolysis, complexation etc. are further subjected to removal through precipitation and adsorption (Imfeld et al., 2009).These chemical processes are prevalent at the rhizosphere and on the substrate’s surface which lead to the short-time retention or long term immobilization of the pollutants (Drizo et al., 1999).Adsorption process largely depends upon the specifications of the substrate and characterization of wastewater .

Chemical composition and ion exchange properties of the substrate play a decisive role in adsorbing process. Total Phosphorus removal is chiefly associated with the adsorption by substrate. Ca/Fe mineral bearing substrates are more efficient in removing TP as compared to gravel based substrates which neither provides organic carbon nor adsorb TP (Comeau et al., 2001).Precipitation is also a dominating chemical process which allows the long term removal of pollutants. These processes are primarily controlled by the pH and redox conditions (Rhue and Harris, 1999). Following table tabulates the different processes involved in the removal of various contaminants from the wastewater through constructed wetlands:

2.10 Leachate treatment through constructed Wetlands

Taking into account the economic viability and environmental aspects of Constructed wetlands also termed as Root-zone bed technology is considered as an ideal option for the treatment of landfill/dumpsite leachate. For an effective and long term leachate treatment it is always recommended to characterize leachate beforehand so that a reasonable constructed wetland design can be developed. Researchers from different countries have conducted lab-scale studies, practically applied this treatment on contaminated sites and reported varying degree of success.

Bulc et al., 2006 conducted an extensive evaluation of seven years on performance efficiency of Constructed Wetlands at Ljubljana’s old sanitary landfill site. Results showed 50% COD removal, 51% NH_4-N removal and 50% removal of phosphates. Iron removal was also evaluated which was reported to 84%. Typically a reasonable removal of organic matter and suspended solids have been reported by Constructed Wetlands. Topal., 2012 carried out a study on removal of various contaminants from the leachate at two different depths through constructed wetlands having *Lemna gibba L.* According to which COD removal from the microcosms having 5cm and

10cm depth was 52.0-58.0% and 51.1-55.4% respectively which showed that depth does not significantly affect the COD removal.

In an another study Akinbile *et al.*, 2012 appraised performance evaluation for three weeks of lab scale subsurface constructed wetlands planted with *Cyperus haspan* in treating landfill leachate from Pulau Burung Sanitary Landfill (PBSL). Results revealed a removal of 59.7-98.8% of TSS; 39.2- 91.8% of COD; 60.8–78.7% of BOD; 7.2-12.4% of pH and 39.3-86.6% of turbidity. All of these removal efficiencies approved *Cyperus haspan* as a promising candidate. As leachates are rich in nutrients at alarming levels sufficient enough to cause eutrophication in the water bodies, studies have also been carried out for the reduction of such eutrophic compounds. Kamarudzaman *et al*., 2011 noted a removal of NH_3-N and PO_4-P through two wetland plant species planted in a HSSF constructed wetlands i.e. Limnocharis *flava* and *Scirpus atrovirens* to be 61.3% (NH_3-N), 52% (PO_4-P), 38.7%(NH_3-N), and 48% (PO_4-P) respectively.

Yalcuk and Ugurlu, 2009 noted the treatment efficiency by three pilot-scale constructed wetlands i.e. VSSF (with and without zeolite layer) and HSSF planted with *Typha lattifolia* in terms of removing COD, Ammonia Nitrogen, Orthophosphate and Fe from landfill leachate. Removal efficiency for $VSSF_1$(with zeolite), $VSSF_2$(without zeolite) and HF was NH_4–N, 62.3%, 48.9% and 38.3%; COD, 27.3%, 30.6% and 35.7%; PO_4–P, 52.6%, 51.9% and 46.7%; Fe(III), 21%, 40% and 17% respectively. High Ammonia removal was observed in $VSSF_1$ as compared to $VSSF_2$ whereas HSSF showed high COD removal.

Chapter 3

METHODOLOGY

3.1 Experimental Setup

In this study, eight laboratory scale constructed wetland systems were established by using plastic tubs (1.5 ft x 1.5 ft x 1 ft). Perforated pipes were laid at the bottom of the tubs with an outlet for the collection of effluent. A valve was fixed with the outlet pipe for timely sampling of the effluent. These tubs were represented as vertical subsurface flow constructed wetland system. Following were the steps which were taken in making VSSF-CW as shown in figure 3.1

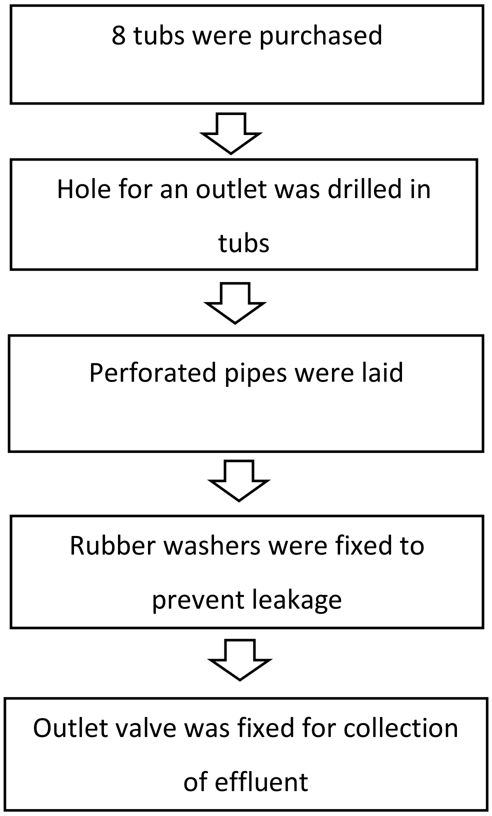


Fig 3.1 Flow chart describing the steps involved in the construction of VSSF-CW

3.2 Soil Analysis

Preliminary soil analysis was made to ensure its suitability for plant growth. Soil was tested for its classification as well as for its pH. Soil classification was made through saturation percentage (Malik et al., 1984). pH was tested by air drying soil about 10 grams were taken out of it in a beaker. 50mL of distilled water was added into soil through graduated cylinder. The mixture was mixed well with the help of a glass rod for 30mins and then allowed to settle. After one hour, pH reading was taken through a Hach SensION1 pH meter (McLean, 1982).Air dried soil of about 10grams was taken in a petri dish. It was dried in oven, at 105 °C overnight. It was removed from oven; cooled in a desiccator for 30 minutes and then re-weighed. Moisture content was calculated using the following relation:

$$\%\, moisture\ in\ soil = \frac{Wet\ soil - dry\ soil}{dry\ soil} \times 100$$

Olsen's test was opted for the analysis of Phosphorus available in soil (Olsen et al., 1954).Whereas Kjeldahl method was used to determine Nitrogen in soil (Ryan, 2008)

3.3 Substrate used in Constructed Wetland

For the collection of effluent as well as for the sake of support for the upper main filter layers, gravel having size of 12-25mm was laid over the pipes up to 3cm length. Above that an even layer of 5cm of sand was spread followed by 10cm thick layer of soil as substrate. These tubs were kept under natural conditions at the backside of the campus. Following diagrams represent design and dimensions of the tubs used in the study:

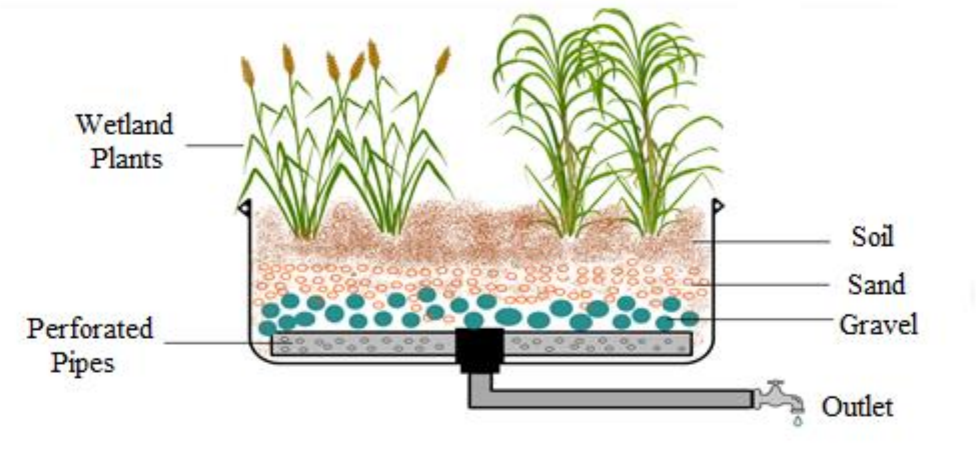


Fig 3.2 Design of Lab-Scale Constructed Wetlands

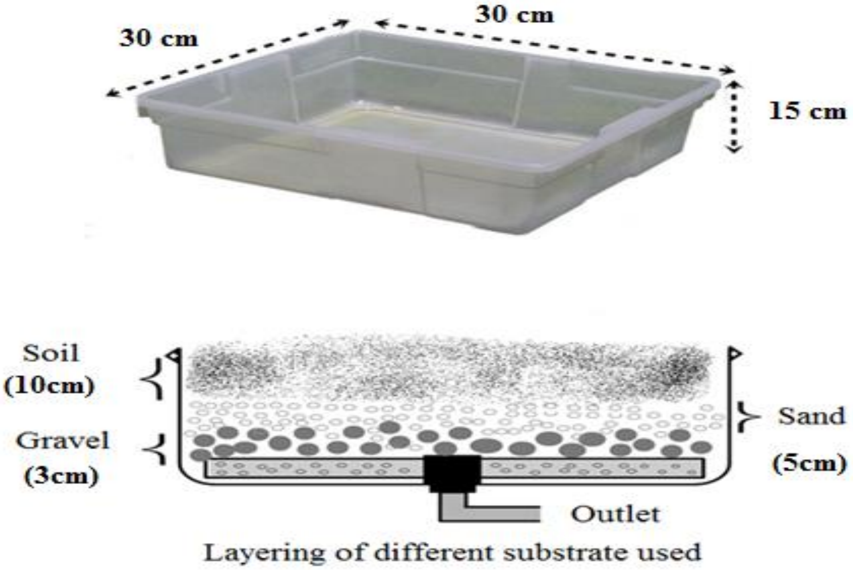


Fig 3.3 Substrate Media used in each Constructed Wetlands

3.4 Plantation

Once the constructed wetlands were established they were implanted with plants (mixed culture). *Phragmites karka* and *Phragmites australis* were selected on the basis of their fast growth and high resistance towards polluted environment. These two are cosmopolitan plant species and can easily be found along the margins of streams and ponds. Twenty four juvenile healthy plants of each specie (average height of 36cm) were transplanted washed with tap water and implanted into each tub, with three plants of each specie in one tub. One of the plant i.e. *Phragmites karka* was transplanted from the NUST H-12 campus where as the other specie i.e. *Phragmites australis* was taken from National Agricultural Research Center (NARC). Efforts were made to collect these plants in their pristine forms. A brief description of these two selected species is given in Table 3.1

Table 3.1 Selected Macrophytes *Phragmites karka* and *Phragmites australis* Profiles

Characteristics	**Phragmites karka Retz.**	**Phragmites Australis**
Kingdom	Plantae	Plantae
Order	Cyperales	Cyperales
Family	Poaceae	Poaceae
Genus	Phragmites Adans	Phragmites
Species	Phragmites Karka Retz.	Phragmites australis Cav.
Common Name	Tall reeds, pit-pit	Common Reeds, Cane grass
Physical Characteristics	- Perennial marshy plant, deep strong root system - Grow as long as 2-6 m - Leaves length range: 30-80cm with 1.27-6cm width - Large quill leafy panicles with spreading branches and attenuated spikelets	- Known as *Phragmites Karka's* temperate counterpart - Grow as long as 20 ft, generally attains 10-12feet - Leaf length: 10-50cm with usual width of 1-5cm - Inflorescence is usually manisfested in form of a reddish or tan brown panicle of spikelets up to 1½ foot long and one-half as much across. Panicle is drooping with dense branchlets.
Habitat	- Found near margins of rivers, streams and ponds (Marwat *et al*., 2007)	- Also found in wet habitats (Saltonstall *et al*.,2004)

3.5 Acclimatization Phase

After the transplantation plants were given tap water to make them acclimatized to this new environment. In the beginning of acclimatization phase plants started to wilt and died gradually but re-sprouting occurred immediately. During acclimatization phase of four weeks, newly sprouted green plants were established. In the course of acclimatization these species showed a slow pattern of growth (Pharagmites karka > Pharagmites australis).

3.6 Preparation of Synthetic Leachate

As the objective of this study revolved around the removal of heavy metals through constructed wetlands in the presence of varying quantities of nutrients and COD levels, various compositions of synthetic leachate were composed in order to avoid likely interferences in the removal process by other potential contaminants which are present in real dumpsite leachate. Synthetic leachate was prepared as described by Madera-Parra et al., (2013). The main parameters in targeted were COD, Nutrients (N and P) and Heavy metals (Pb and Fe). Glucose ($C_6H_{12}O_6$), Potassium Dihydrogen Phosphate (KH_2PO_4), Ammonium Chloride (NH_4Cl), Lead acetate $Pb(C_2H_3O_2)_2$, and Ferrous Sulphate, Heptahydrate (FeSO4.7H2O) were used as a source of Organic Matter, Phosphorus, Nitrogen, Lead, and Iron respectively. Three various compositions of synthetic leachates were prepared. Following table shows the various compositions of synthetic leachate:

Table 3.2 Amounts of Chemicals used in Preparation of Synthetic Leachate

Constituents	CW_1	CW_2	CW_3	$CW_{control}$
NH_4CL(mg/L)	154	223	296	nil
KH_2PO_4(mg/L)	72.38	108	144	nil
$C_6H_{12}O_6$(mg/L)	880	1320	1760	1320
$Pb(C_2H_3O_2)_2$(mg/L)	4.71	4.71	4.71	4.71
$FeSO_4.7H_2O$(mg/L)	49.82	49.82	49.82	49.82

3.7 Application of Synthetic Leachate

Each individual 2L composition was prepared by carefully weighing the chemicals and they were mixed with tap water in a large beaker and then the synthetically produced leachate was fed to each set of constructed wetlands immediately after preparation in order to avoid potential organic decomposition and precipitation of salts. Experimental units were divided into four groups which were watered with various compositions of synthetic leachate. Duplicate of each experimental setup were maintained with a set of controls which were only given heavy metals along with COD of 1200mg/L without nutrient supplements. Details are given in Table 3.3:

Table 3.3 Composition of Synthetic Leachate for all the Constructed Wetlands

Components (mg/L)	CW_1	CW_2	CW_3	$CW_{Control}$
Fe	10	10	10	10
Pb	3	3	3	3
Nitrogen	50	75	100	Nil
Phosphorus	50	75	100	Nil
COD	800	1200	1600	1200

3.8 Plant Growth

Growth characteristics of plants were monitored during the study which comprised of 8 weeks (56 days). Representative plants from each experimental setup were observed for their individual height, number of new leaves and toxicity symptoms (once in a week). Every week photos were taken of the plants per treatment. Individual height was measured through measuring tape. A plant notebook was maintained in which additional leaf formation along with their length, stem diameter, toxicity symptoms and total biomass were written down once a week except for total biomass which was weighed after the harvest of plants.

3.9 Effluent Analysis

Effluent from all the sets was sampled twice a week. Samples were subjected to heavy metal as well as Chemical Oxygen Demand (COD) analysis. COD was measured according to Closed Reflux Method where as Heavy metals were measured according to Direct Air Acetylene Flame Method through atomic absorption spectrophotometer as mentioned in Standard Methods and Procedures for Waste water Examination (APHA, 2012). In case of heavy metal analysis efforts were made to analyze the collected samples immediately but if the experiment was delayed for any reason then few drops of HNO_3 were added to avoid precipitation of metals.

3.9.1 COD analysis in effluent

Chemical oxygen demand (COD) is defined as the organic load which cannot be degraded biologically. Usually a strong oxidizing agent is used to oxidize organic and inorganic compounds including NH_3 and NO_2^- .COD measurement is considered one of the important parameter in evaluating and determining the organic load contamination. Closed reflux method was adopted to measure the COD of effluent. Calculated amounts of Potassium Dichromate (K_2Cr2O_7) and Sulphuric Acid (H_2SO_4) were added to sample and then were subjected to block digester for digestion at 150˚C for two hours. After the digestion,the remaining reduced $K_2Cr_2O_7$ was titrated with ferrous ammonium sulphate by using digital titrator ie Titroline easy to determine the amount of K2Cr2O7 consumed and the oxidizable matter measured in terms of the oxygen equivalent. Following formula was applied to calculate COD:

$$\text{COD as mg } O_2/L = \frac{(A-B)\times M\times 8000\times D.F}{\text{mL of sample}}$$

where A stands for the amount of FAS used for blank B is the amount of FAS used for sample. M is the molarity of FAS.8000 is the milli-equivalent weight of oxygen × 1000mL/L.Where D.F stands for Dilution Factor.

3.9.2 Heavy Metal Analysis in effluent

The effluent loads were analyzed for Fe and Pb through atomic absorption spectroscopy (AAS). Out of 20ppm stock solution desired standards were prepared for Iron (Fe) and Lead (Pb). Every time fresh working standards were used.

3.10 Harvesting Of Plants

At eighth week plants were ready to be harvested. Plant samples from each set were uprooted cleansed with tap water segregated into shoots and roots. Roots were carefully rinsed twice first with tap water and then distilled water. Later they were blotted dry on paper. Segregated plants were marked and then immediately transferred to lab to weigh fresh biomass by using electronic weighing balance. Then samples were subjected to oven at 80˚C for 48 hours. Later their dry biomass was also weighed. Then samples were ground to powder by using ceramic pestle and mortar and sieved through a mesh of 2mm nylon sieve and placed in plastic sample bags.

3.11 Digestion of Plant Samples

Approximately 0.1g of plant material was weighed and transferred to 50ml volumetric flask. On the other hand Nitric Acid (HNO_3) and Percholoric Acid ($HClO_4$) mixture (2:1) was prepared freshly and added into each 50ml volumetric flask. Then the flasks were placed on a hotplate plate and the temperature was gradually increased from 120˚C to 200˚C to allow effective digestion. Digestion of samples was performed under a fume hood in order to ensure safety. It took about 20 to 45 minutes when HNO_3 volatilized as nitrous oxide fumes, and then white fumes of Perchloric acid came out from the flask. The solution in flask was white in colour at that stage. After digestion, the flasks were removed from the hot plate allowed to cool and 50ml of distilled water was added. The plant digested material was filtered through Whattmann No. 42 filter paper, refrigerated at 4˚C for further analysis.

3.12 Analysis of Iron (Fe) and Lead (Pb) in plant tissues

The digested plant samples were analyzed for Iron (Fe) and Lead (Pb) through Atomic Absorption Spectrophotometry (Phoenix-986 AAS) with air/acetylene burner. Standards of 2ppm, 5ppm, 10ppm and 15ppm were prepared for Iron (Fe) whereas 2ppm, 4ppm, 6ppm, 8ppm and 10ppm were prepared for Lead. The lamp wavelength for Iron was 248nm whereas for Lead it was 283nm. Each sample was run into triplicate. Results are shown as mean ± Standard error.

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Soil Analysis

Texture of soil used in experiment was silt loam. Soil pH was tested to corroborate its suitability for plant growth it came out to be 8.64 which was appropriate for the plant growth. Nutrient analysis reveal 8.2mg/kg and 0.7mg/kg of Phosphorus and Nitrogen respectively.

4.2 Plant Growth Response

Figure 4.1 and 4.2 illustrated the effect of synthetic leachate having various compositions on physical growth of *Phragmites karka* and *Phragmites australis* respectively. Both the plants showed reasonable adaptability for the constructed wetland design used in this study, as they showned spontaneous growth in their shoot system and were able to withstand all the desired operating environmental conditions applied in this study.

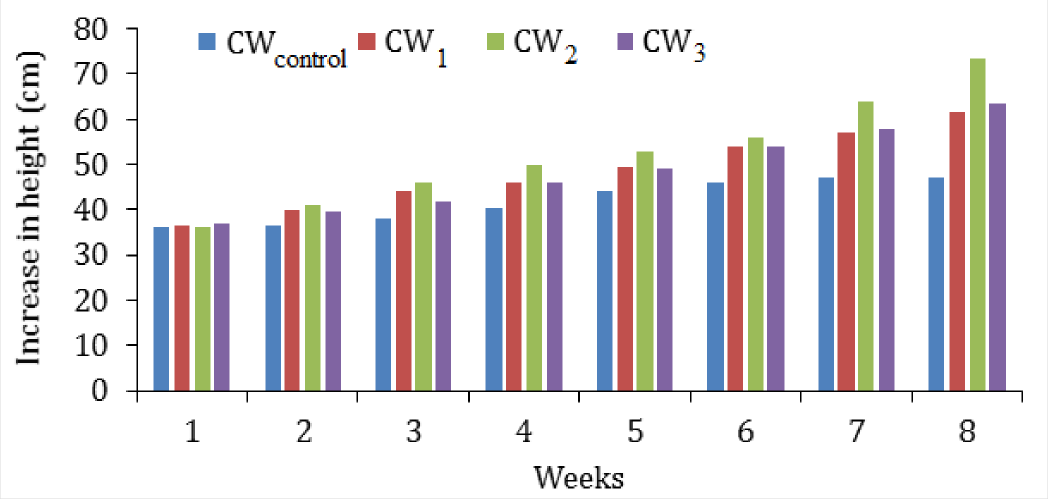


Figure 4.1: Increase in height of *Phragmites karka*

During the first week of the study, growth of *P. karka* was visually similar for each CW but with the passage of time each wetland showed a different shoot growing trend as shown in figure 4.1

In these eight weeks of study total shoot increment of 11, 25, 37, and 26.5cm was showed by $CW_{control}$, CW_1, CW_2 and CW_3 respectively by the *P. karka*.CW_2 showed the highest increment in height of shoots as compared to other treatments and especially control (with 0 additional nutrient supplements). Nutrients play a vital role in the growth of plants. Wong et al., 1997 studied that constructed wetlands planted with *Aegiceras corniculatum* which were fed with high nutrient concentrations were more immune towards toxicity and showed a better plant growth compared to the plants fed with less nutrient concentrations. For CW_3 *karka* growth was less as compared to CW_2, this may be due to high nutrient loading then the plant demand which resulted into algal growth in the system.

Shoot length of *P. australis* was higher as compared to *P. karka* plant. Increment of 38.3, 48.1, 55.7 and 57.3cm for $CW_{control}$, CW_1, CW_2 and CW_3 respectively was observed for *P. australis* plants in this study. Referring to Figure 4.2 similar to *P. karka* plant, *P. australis* plants which were fed with nutrient rich leachate showed higher shoot increment. CW_2 and CW_3 showed nearly equal shoot growth which depicts that *P.australis* can withstand concentrated leachate.

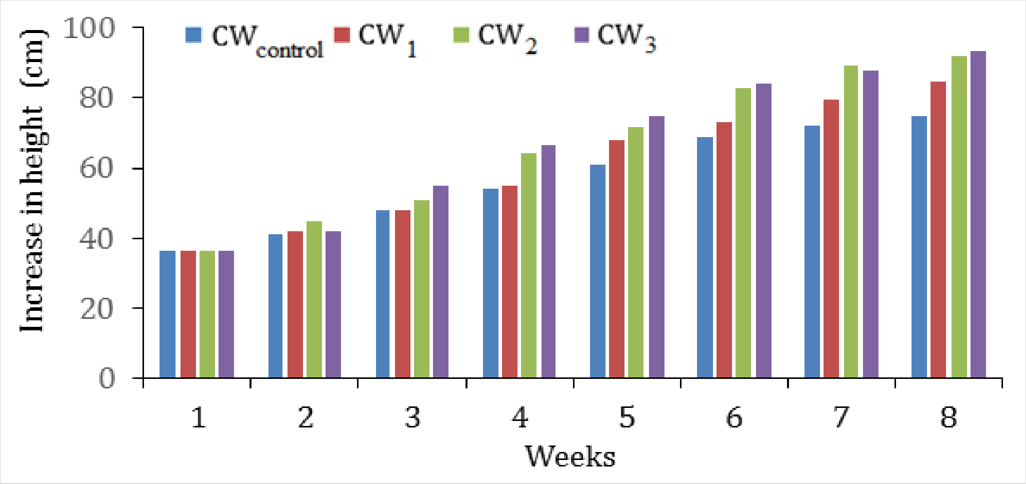


Figure 4.2: Increase in height of *Phragmites australis*

Limited growth by both plants in control type was contributed by the fact that it was provided with heavy metals including 1200mg/L of Organic Matter without any nutrient supplements. In a study by Verma et al., 2014 found that *P. karka* which was fed with arsenic as high as 1400ppb without any nutrient supplements showed stunted growth. Overall growth rate was higher with prominent increment in height in all three sets as compared to control.

4.2.1 Phenotypic response

In the third week both the species started to manifest clear signs of phytotoxicity which included yellowing of plants (Chlorosis).Tillers were observed in almost all units except for control. Mealy plum aphid *Hyalopterus pruni* (Geoff) infestation was also observed in Phragmites australis which started at the 3rd week of treatment. *P. australis* is a natural host of *H. pruni(* Tscharntke , 1989). Infestation increased as the weeks passed by. Control group was the first one to display toxicity symptoms followed by CW_3, CW_1 and CW_2.

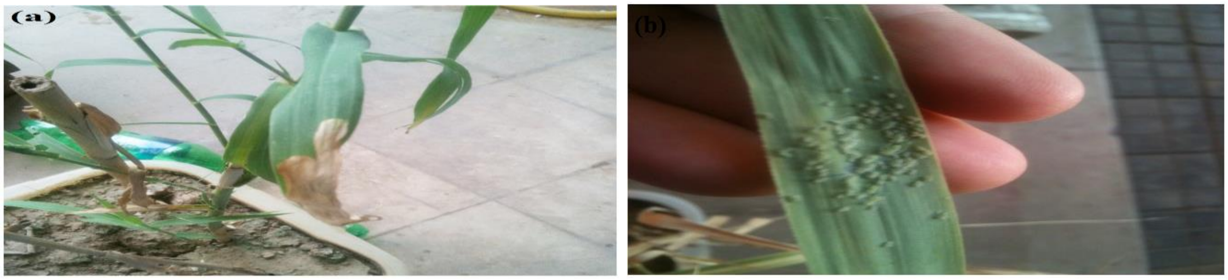
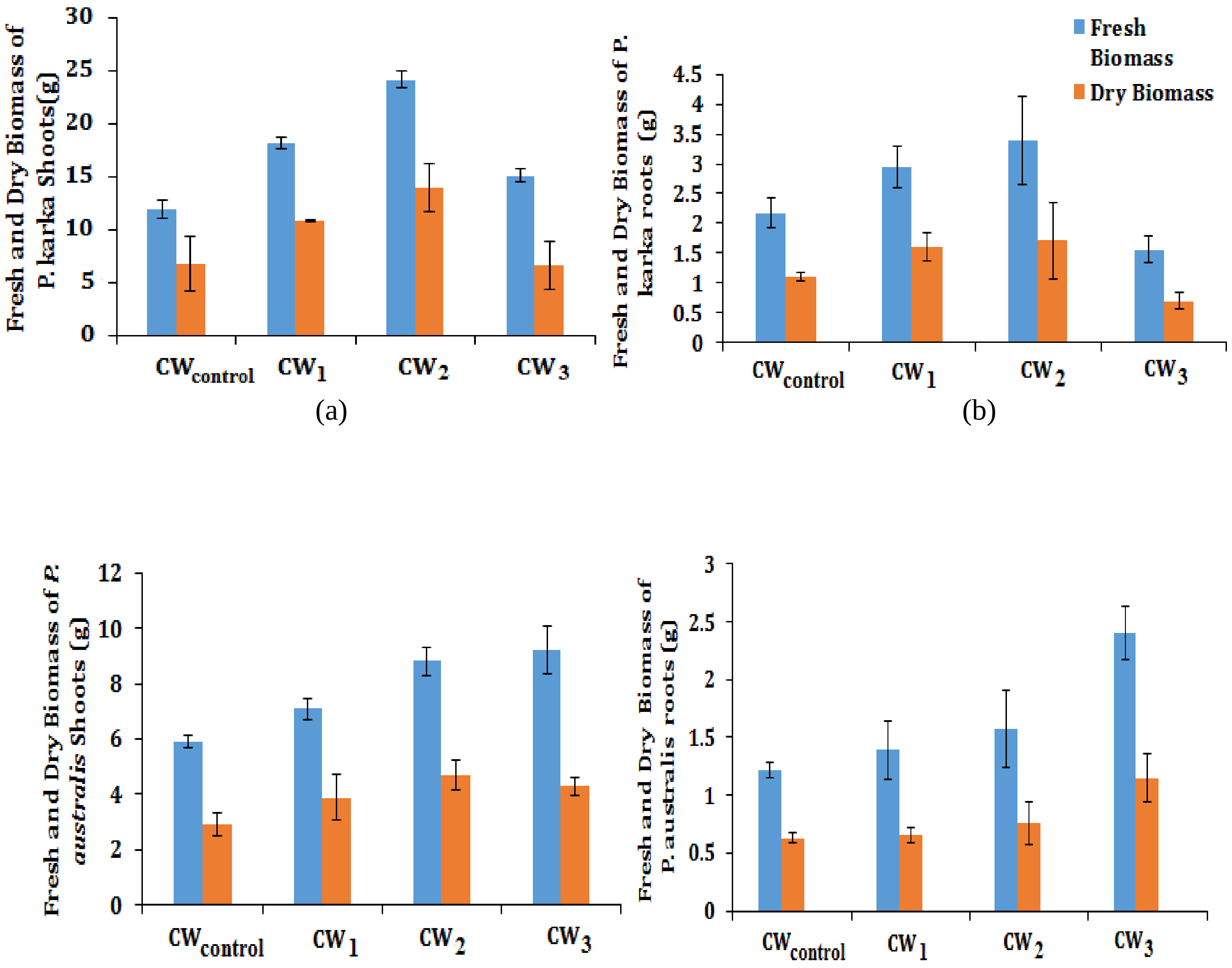


Figure 4.3 (a) Chlorosis occurring on P.karka leaf (b) Aphid infestation on *P. australis*

4.2.2 Fresh and Dry biomass of plants in response to various compositions of synthetic leachate

In spite of the presence of heavy metals (Fe and Pb) and high content of organic matter growth of *Phragmites karka* and *Phragmites australis* was enhanced due to presence of additional nutrients which were given at three different concentrations. Standard deviation and mean was calculated for the precision of results.



(a) (b)

(c) (d)

Figure 4.4 (a) Fresh & dry biomass of *P. karka* shoots (b) Fresh & dry biomass of *P. karka* roots (c) Fresh and dry biomass of *P. australis* shoots (d) Fresh and dry biomass of *P. australis* roots

Referring to Figure 4.4 (a) *Phragmites karka* shoots planted in CW_1, CW_2 and CW_3 showed 51, 101 and 26% increase in fresh biomass as compared to $CW_{control}$ respectively .Whereas shown in 4.4 (b) *P. karka* roots of CW_1 and CW_2 manifested 33.3 and 55% increase when compared to $CW_{control}$ respectively . *P. karka* roots planted in CW_3 showed anomaly which was a decrease of 28% as compared to $CW_{control}$. This decline can be attributed to the concentrated levels of contaminants introduced through synthetic leachate into CW_3 which could have proved detrimental for the below ground biomass of *P. karka.*

Figure 4.4 (c) and (d) present *P. australis* shoots and roots fresh and dry biomass respectively. There has been an increase noted in the root/shoot biomass of *P. australis* against the ascending order of nutrients and organic matter concentrations along with similar doses of Iron and Lead . This increasing trend proved that *P. australis* can grow well in high contaminating levels. It has been critically observed that the fresh biomass of shoots of *P. australis* grown in CW_1 , CW_2 and CW_3 increased 20.3, 49.12 and 55.9% respectively as compared to control group which was not given any additional nutrients.While the fresh biomass of *P. australis* roots accounted for 13.9, 29.5, 96.7% respectively. In another study wastewater from a primary pond receiving wastewater from the pig farm as well as municipal sewer water from the housing at the food processing industry was used it contained 478mg/L and 135mg/L of Total Nitrogen(TN) and Total Phosphorus (TP) was treated by *Sesbania sesban* planted constructed wetlands. *S. Sesban* responded well towards nutrient rich wastewater and produced high biomass (Dan et al.,2011).

4.3 Metal Accumulation in Roots/Shoots of Plants

One of the salient feature of wetland whether natural or constructed is the removal of contaminants through biological means. Shelef et al., 2013 outlined plant uptake as one of the widely recognized biological process for the removal of heavy metals. Hence analysis of the roots and shoots were done at the end of experiment in order to appraise the quantities of heavy metals sequestered in plant tissues. Following graphs shows accumulation trends of heavy metals by selected macrophytes:

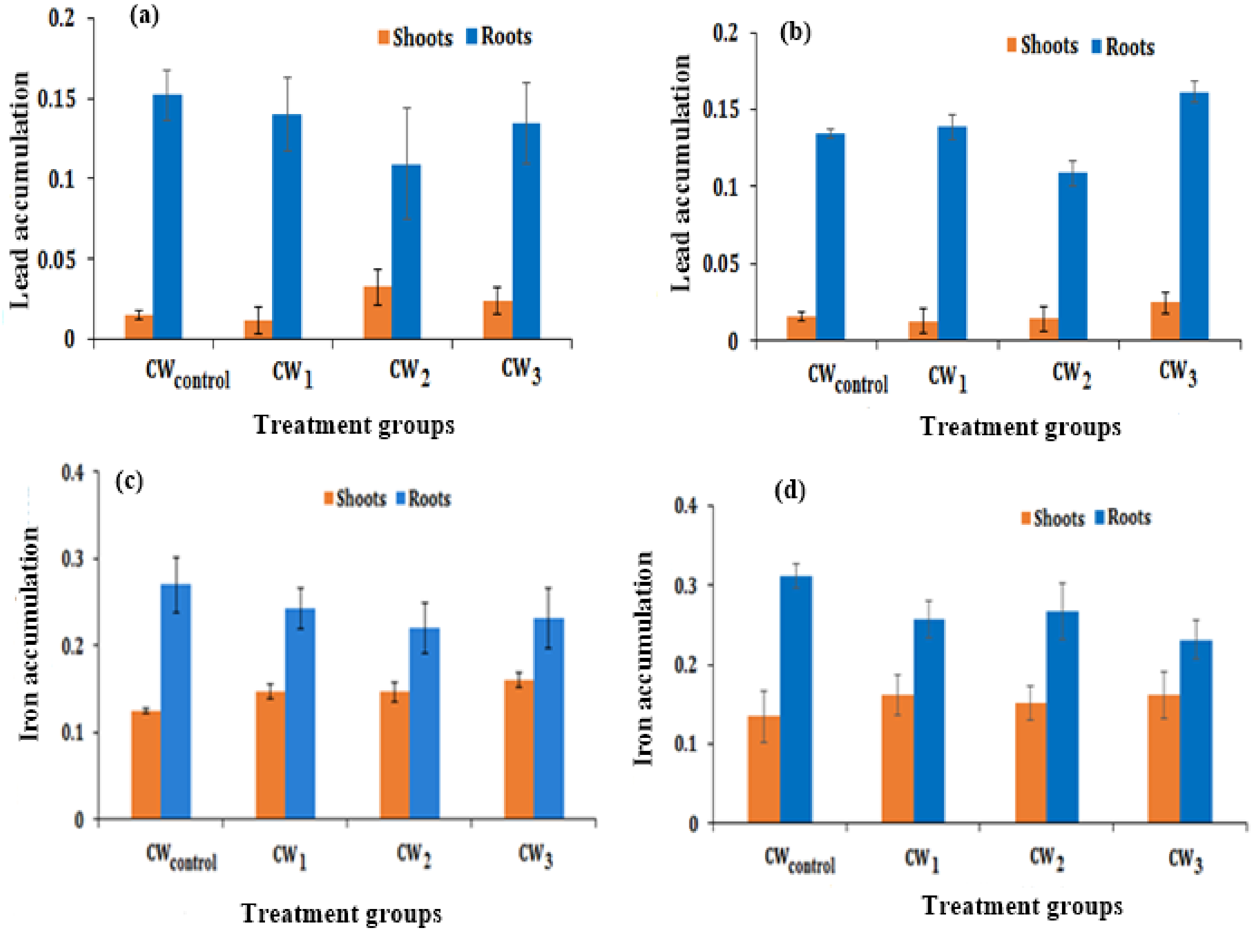


Figure 4.5 (a) Lead uptake by *P.karka* (b) Lead uptake by *P.australis* (c) Iron uptake by *P.karka* (d) Iron uptake by *P.australis*

Referring to Figure 4.5 (a) and (b) which represent Lead and Iron uptake by *Phragmites karka* respectively. Lead accumulation in roots of *P.karka* was calculated to be 0.152, 0.14 , 0.109 and 0.135ppm respectively. Minor amounts of lead were also detected in the shoots of *P.karka* plants that were given different treatments. P.karka shoots planted in $CW_{control}$, CW_1, CW_2 and CW_3 accumulated 0.015, 0.012, 0.032 and 0.024ppm respectively. Iron accumulation in roots of *P. karka* was gauged as 0.27, 0.243, 0.220 and 0.231ppm respectively where incase of shoots of *P.karka* it was 0.13, 0.15, 0.147 and 0.161ppm in $CW_{control}$, CW_1, CW_2 and CW_3 respectively.

Figure 4.5 (c) and (d) illustrates lead and iron accumulation in *P. australis* respectively. Lead accumulation in *P. australis* roots at different treatments came out to be 0.135, 0.148, 0.11 and 0.161ppm.Very minute quantities of lead were detected in *P. australis* shoots. Fitzgerald et al., 2003 found that copper accumulates primarily in the roots of monocots and dicots, while lead accumulates mainly in the roots of monocots but in the shoots of dicots. Since lead is a toxic and a non-essential metal, so macrophytes use their root epidermis as barriers to hinder upward translocation of lead (Weis and Weis, 2004). In case of iron uptake in P. australis it was detected in almost equal amounts in above and below ground tissues.

Tangahu et al., 2011 reported that uptake of metals by plants depends upon the bioavailability of metal in water phase which is further dependent upon the retention time of metals. In terms of lead accumulation there was significant difference observed between roots and shoots of both the species. But on the whole lead accumulation trend remained significantly same for all the sets. Iron uptake was significantly higher as compared to lead uptake in both the species. Kamal et al., 2004 explained that iron is an essential micronutrient (Fe^{+2}) which can easily be absorbed and translocated to aboveground biomass. Rascio and Izzo, 2012 reviewed extensive literature and reported that heavy metals are not only retained in the roots but also transferred to the shoots and deposited in the leaves, at concentrations 100–1000- fold higher than those found in non-hyper accumulating species. Mengzhi et al., 2009 stated that area around plant root ie rhizosphere and substrate absorb ionic heavy metals. The root epidermis serves as a barrier against the transportation of lead to aboveground tissues.As the plants were not in direct contact with wastewater ie anchored into soil for that they sequestered low quantities of metals in their tissues. High doses of nutrients played a role in alleviating the metal stress as proved in plants planted in CW_1, CW_2 and CW_3 that sequestered heavy metals in their tissues without the production of any toxicity or reduction in growth. Control was the first one to manifest signs of toxicity as it was not given additional nutrients. Statistically non-significant differences in terms of metal uptake at varying levels of nutrients and organic matter were reported when the probability of the result assuming the null hypothesis ($p < 0.05$).

4.4 Results of effluent Analysis

VSSF Constructed Wetlands showed reasonable efficiency in terms of removing Chemical Oxygen Demand (COD) and heavy metals from the synthetic leachate. After the commencement of 4th week CW_3 started to exhibit signs of being choked. A thick layer of algae was formed in the CW_3 which obstructed the water passage through the system for that very reason no effluent was collected at outlet after 4th week

4.4.1. COD Removal by the Constructed Wetland Systems

Constructed wetlands have proven their worth in removing organic pollution from different kinds of wastewater. Organic content is largely consumed by the various kinds of bio-chemical reactions taking place within a constructed wetland system.

Figure 4.10(a, b, c and d) shows the COD removals by all the constructed wetlands used in the study with an average COD removals of 37.3, 42.7, 44.9%, and 18.9% for $CW_{Control}$ CW_1, CW_2, and CW_3 and respectively.

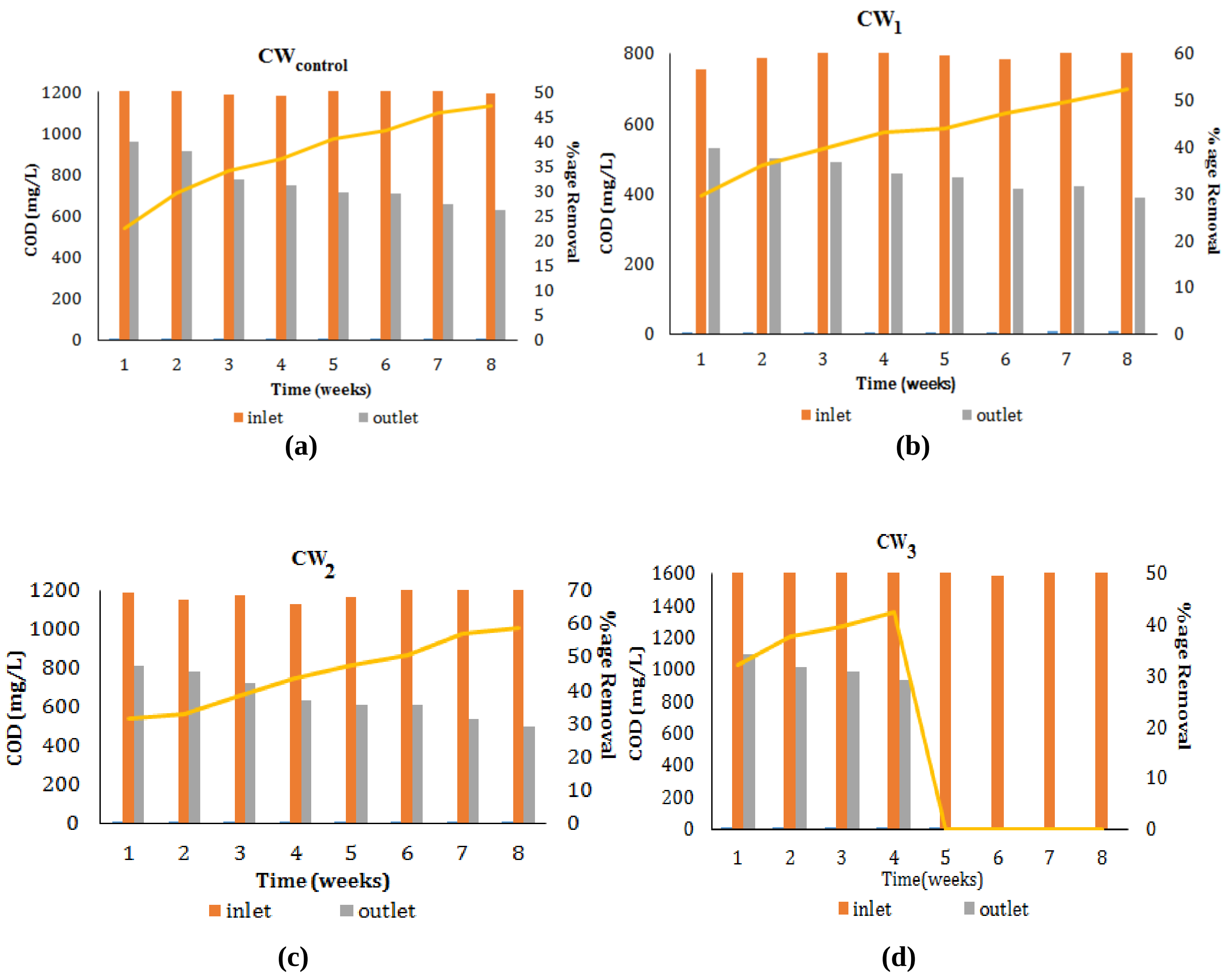


Figure 4.6 COD removal under various treatments observed during study period (a) $CW_{control}$ (b) CW_1 (c) CW_2 (d) CW_3

Removal efficiency for CW_2 was higher than CW_1 while removal efficiency for Control was lower as compared to other CWs. On the other hand CW_3 also progressed with an increasing removal trend but later it choked due to nutrient enrichment and possible high organic loading. Similar choking problem was reported by Langergraber et al.,2007 and suggested applying wastewater with less nutrient and organic content into VSSF-CWs .CW_2 showed better removal then CW_1 because CW_2 was fed with synthetic leachate containing more nutrient content than the

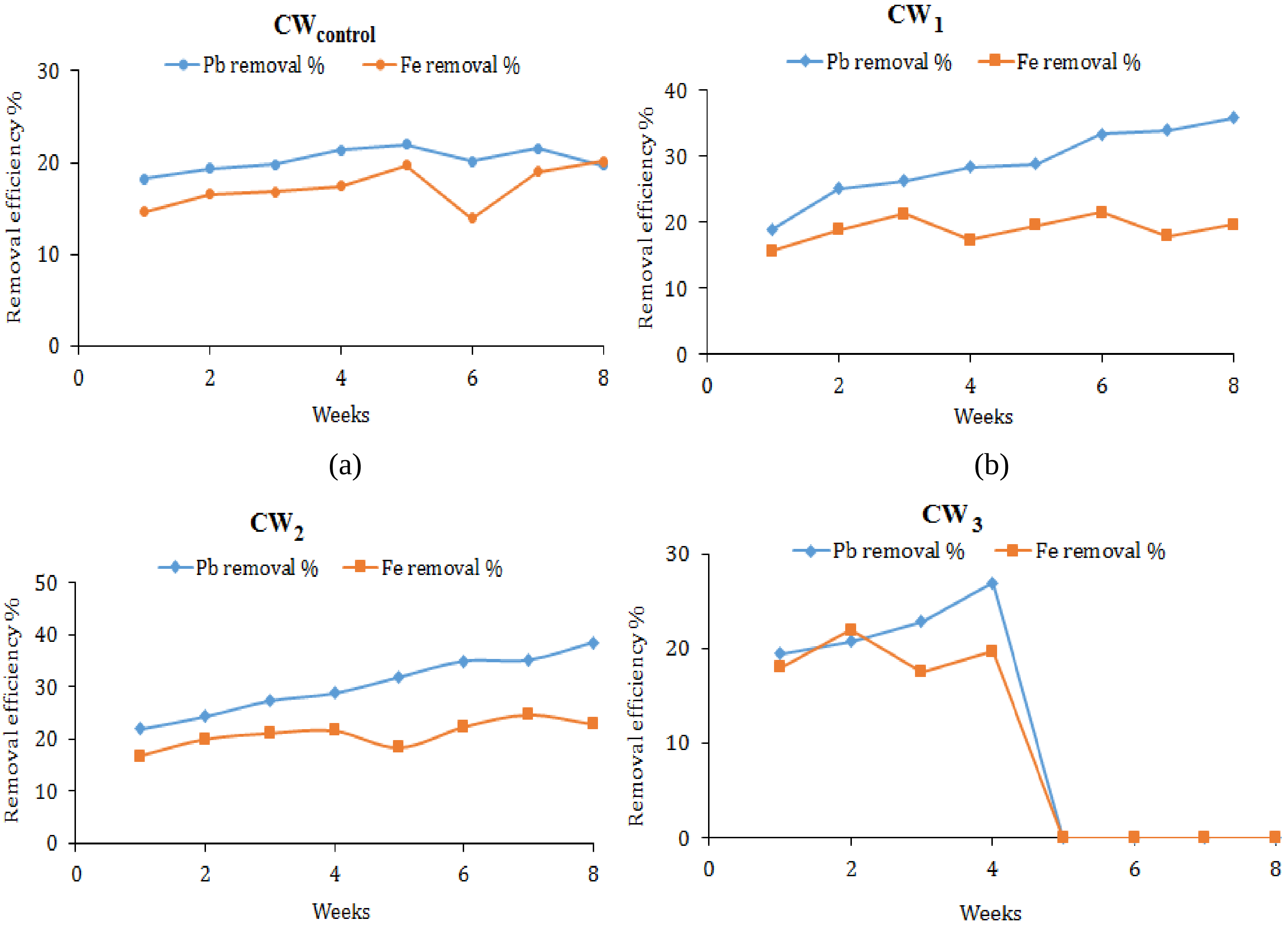
CW_1 this was proved by comparing the results of CW_1 and CW_2 with control, removals were higher in case of CW_1 and CW_2 because Control was not supplied with any additional nutrient supplements. This trend was also observed by Rodriguez-Caballero et al., 2014, in his study organic degradation was increased with the addition of Nitrogen and Phosphorus in pilot scale constructed wetland systems.In constructed wetland oxygen is diffused into substrate through the roots of the plants which activates the aerobic microbial activities which accelerates the microbial consumption of organic matter. Under such conducive conditions biofilms are formed which encourages high rates of organic degradation thus leading to reasonable removal efficiency with the passage of time. Lim et al., (2003) proved that biofilm formed in the substrate plays an important role in the removal of organics from the constructed wetlands. A strong biofilm might be developed in CW_2 due to high nutrient dosage so it may also be the reason for high COD removals in it as compared to $CW_{control}$ and CW_1.

In case of CW_3, there was no effluent at outlet with the commencement of 5th week because the system was clogged. This occurred due to eutrophication process as CW_3 was fed with high phosphorous and ammonium-nitrogen values which cause sufficient algal growth on the substrate used in CW_3, this algae was main factor to clog the system as it obstructed the water passage. So it was concluded that while dealing with the leachates with high nutrients values, one should have to follow proper maintenance precautions, proper cleaning and substrate replacement is required under high eutrophicated conditions.

4.4.2 Heavy metal removal from Synthetic Leachate

Atomic Absorption Spectrophotometer results revealed the initial and final concentration results of 8 weeks (56days) for Iron (Fe) and Lead (Pb) from four variant synthetic leachates. Figure 4.7 (a, b, c and d) depict graphical representation of removal efficiencies for lead and iron.

Efficacy of all the sets of constructed wetlands for the heavy metal removal increased gradually. As compared to iron high removal efficiency for lead was observed. For lead (Pb) CW_1 started off with a removal of 19% which culminated at 35.8% on 8th week. Whereas removal range of 21.9-38.6% was observed in CW_2. CW_3 initiated with a removal rate of 19.4% which ceased at 26.9% on 4th week because of the clogged substrate. Control showed an average removal of 20.2% for lead.



(a) (b)

(c) (d)

Fig 4.7 Iron and Lead removal efficiency (a) $CW_{control}$ (b) CW_1 (c) CW_2 (d) CW_3

Efficacy of all the sets of constructed wetlands for the heavy metal removal increased gradually. As compared to iron high removal efficiency for lead was observed. For lead (Pb) CW_1 started off with a removal of 19% which culminated at 35.8% on 8th week. Whereas removal range of 21.9-

38.6% was observed in CW_2. CW_3 initiated with a removal rate of 19.4% which ceased at 26.9% on 4th week because of the clogged substrate. Control showed an average removal of 20.2% for lead.

Iron removal efficiency for all the constructed wetlands remained almost the same. For CW_1 it started off with 15.7% and on 8th week it went upto 19.7%. Almost similar results were exhibited by CW_2 which showed 16.8% in the first week and 22.8% in the 8th week. Data for four weeks of CW_3 was collected which showed 18% removal efficiency in first week and ceased working after 4th week by attaining as high as 19.6%. Control was also not so different from other sets it also showed an average of 17.2%. A very fluctuated data for iron removal was observed in all constructed wetlands. It can be attributed to the fact that we used metallic valves and probably due to rusting the iron could have leached into the efflux thus giving low removal efficiency.

A diverse range of reactions take place immediately after the addition of synthetic leachate into the constructed wetlands. This may include metal complexation with organic/inorganic ions present in substrate pore water which ultimately leads to the reduction in amount of soluble metal ions (McLaughlin, 2001). Formation of insoluble heavy metal compounds immobilizes the metal and reduces their bioavailability (Ali et al., 2013).

Cao et al., 2001 reported that the main mechanism of Pb immobilization in soil is via dissolution of P and/or meta-stable Pb compounds and the subsequent precipitation of pyro morphite-like minerals. Increased removal efficiency of lead can be attributed to this reason.

Metal ions present in synthetic leachate used in our experiment were present as cations when entering the substrate pore water. Simultaneously, solution metal concentrations may decrease through adsorption or precipitation processes. Adsorption processes are due to an electrostatic bond between the metal and the charged surfaces in substrate. Thus, cationic metals are sorbet most strongly at high pH (McLaughlin, 2001).Metal cations are removed from solution by precipitation reactions which form new solid phases, usually in association with a corresponding anion already present in the solution. The longer the metal is in contact with soil, the greater is the strength of the bond formed. This increasing strength of bond may be due to diffusion of metal into micro pores on the substrate surface**.**

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

Experiments comprising of eight weeks revealed that the overall heavy metal uptake by the two selected wetland species *Phragmites australis* and *Phragmites karka* from the synthetic leachate which contained varied amounts of nutrients and COD was of 1-2%. This minor uptake was observed in all the groups including $CW_{control}$. In all sets Lead was detected in more amounts in roots of both species as compared to shoots wherein Iron being a micronutrient was present in almost equal amounts in both roots and shoots. Among all the groups CW_2 stood to be the efficient in terms of providing highest lead and iron removal efficiencies 30 and 21% respectively. An average COD removal of 42.7, 44.9, 18.9 and 37.3% for CW_1, CW_2, CW_3 and $CW_{Control}$ respectively was observed. It was observed that as the system matured it showed efficient results in removing organic matter from the synthetic leachate. *Phragmites australis* planted in CW_3 which was provided with 100mg/L of N and P and 1600mg/L of COD showed the highest increase in shoot length (93.5cm) where *Phragmites karka* showed highest shoot length in CW_2 .Both species showed stunted growth in control which was devoid of additional nutrients. *P.australis* grown in CW_3 produced 56 and 98% more shoots roots fresh biomass as compared to $CW_{control}$ whereas P.karka planted in CW_2 produced 101 and 55% shoots roots fresh biomass. An anomaly was observed in CW_3 *P.karka*'s roots fresh biomass which showed a decreased biomass of -26% as compared to $CW_{control.}$ An increase has been noted in the root/shoot biomass of *Phragmites australis* against the ascending order of nutrients and organic matter concentrations. This increasing trend proved that *Phragmites australis* can grow well in high contaminating levels as compared to *Phragmites karka.*

5.2 FUTURE PERSPECTIVE

Pakistan being a developing country with limited water supply requires a cost effective as well as energy efficient wastewater treatment methodology where constructed wetlands fit the bill. This solar powered wastewater treatment system is an emerging field in Pakistan which is yet to be explored for its ability to treat various kinds of wastewaters. Hence based on my research following recommendations are proposed to extend this study further:

1. Planted and unplanted constructed wetlands should be compared in order to appraise the role of plants in removing pollutants from the wastewater.

2. Studies should be conducted for varying hydraulic retention time (HRT), kinds of plants, type and size of constructed wetlands system in order to gauge its contaminant removal efficiencies.

3. Heavy metals and nutrient accumulation in soil sediments can also be tested which will shed light on role of soil in sequestering contaminants. Different substrate conditions can be tested for their removal efficiencies for various contaminants.

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