WASTEWATER TREATMENT BY PHYTOREMEDIATION IN CONSTRUCTED WETLAND

A Comparative Study Using *Chrysopogon zizanioides* and *Phragmites karka*

Nandani Pari Ghimire

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RECOMMENDATION

This is to certify that Mr/Ms Nandani Pari Ghimire has completed this thesis entitled "Waste Water Treatment by Phytoremediation in constructed wetland: A comparative study using *Chrysopogon zizanioides* and *Phragmites karka*" as a partial fulfillment of the requirement of MSc in Environmental Science under my/our supervision and guidance. This is his/her original work and to the best of my/our knowledge, this thesis work has not been submitted for any degree in any institutions.

We/I therefore, recommend the dissertation for acceptance and approval.

Dr. Bhoj Raj Pant

Dissertation Supervisor

Nepal Academy of Science and Technology

Mr. Sunil Babu Khatri

Dissertation Co-Supervisor

Faculty, Khwopa College

LETTER OF APPROVAL

On the recommendation of supervisor Dr. Bhoj Raj Pant this dissertation submitted by Nandai Pari Ghimire entitled " Waste Water Treatment by Phytoremediation in constructed wetland: A comparative study using *Chrysopogon zizanioides* and *Phragmites karka*" is approved for the examination and submitted to Khwopa College for the partial fulfillment of the requirements of MSc in Environmental Science.

....

Kamal Raj Gosai

Incharge, Department of Science,

Khwopa College

LETTER OF ACCEPTANCE

This dissertation entitled **"Waste Water Treatment by Phytoremediation in constructed wetland: A comparative study using *Chrysopogon zizanioides* and *Phragmites karka*"** submitted by **Ms. Nandani Pari Ghimire** is examined and accepted as a partial fulfillment of the requirement MSc. degree in Environmental Science.

....
Mr. Roopak Joshi
Principal
Khwopa College

....
Mr. Kamal Raj Gosai
In-charge, Dept. of science
Khwopa College

....
Dr. Bhoj Raj Pant
Dissertation Supervisor
Nepal Academy of Science & Technology

....
Mr. Sunil Babu Khatry
Co-supervisor
Faculty, Khwopa College

....
Asst.Prof. Ramesh Raj Pant
External Examiner
Central Department of Environmental Science
Tribhuvan University

....
Prof. Dr. Siddhi Bir Karmacharya
Chairman, Research Committee
Khwopa College

Date:

DECLARATION

I hereby declare that the work presented in this dissertation is a genuine work done originally by me and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

……………………..

Nandani Pari Ghimire

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ABSTRACT

Constructed wetland technology has been applied for the wastewater treatment. The study was carried out in experimental design wetlands (horizontal type) at Khwopa college premise to treat the wastewater from kitchen, laboratory and toilet for the six-month duration. The main objective of the study was to find out the efficiency of constructed wetland by using Vetiver (*Chrysopogon zizanioides*) and common reed (*Phragmites karka*) in terms of set design parameters (BOD_5, COD, NO_3-N, TP, Chloride, CO_2, Total coliform) and determine the morphological changes in the reeds. Besides, the changes in soil macronutrient changes were also studied in terms of OC, total nitrogen and phosphorous. Two individual and one mixed ponds were used for the selected reeds. The results showed that on the sixth month the overall concentration of BOD_5, COD, NO_3- N, TP, Free CO_2 and Chloride content in the effluent after treatment were reduced by 84.70%, 77.64%, 90.98%, 87.5%, 36%, 81.13% by *Chrysopogon zizanioides* pond 61.53%, 34.22%, 81.88%, 55%, 12%, 52.83% by *Phragmites karka* pond, 76.92%, 36.08%, 84.13%, 60%, 62.5%, 20% by the mixed pond and 38.46%, 25.49%, 29.97%, 32.5%, 4%, 26.41% by the control respectively at their outlets. The percent organic carbon in the soil was decreased in Vetiver pond (3.13% to 0.22%), mixed pond (2.90% to 0.95%), control pond (3.13% to 1.47%) and Common reed pond (2.54% to 1.51%). The Nitrogen in the soil also showed decreasing value from 0.24%, 0.20%, 0.22% and 0.24% in *Chrysopogon zizanioides* pond, *Phragmites karka* pond, mixed pond and control pond before plantation to 0.04%, 0.13%, 0.09% and 0.12% after six months respectively. Likewise the available phosphorous in the soil was also found decreased. Among the morphological, physical, chemical, microbial and soil parameters *Chrysopogon zizanioides* showed maximum height increment, reduced value of physical and chemical parameters of soil as well as wastewater compared to *Phragmites karka* pond, mixed and control pond along with the plants growth. Comparing the relationship among those parameters it was found that wastewater after passing through soil and plants, the toxic chemicals were absorbed and transformed to useful parameters by soil microorganisms and plant roots which were either used as food for plants and soil organisms or released back to the atmosphere by the mechanism of gaseous cycle.

Keywords: Constructed wetland; Chrysopogon zizanioides; Phragmites karka; Designed parameters; Morphology.

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ABBREVIATIONS AND ACRONYMS

ABR	Anaerobic Baffled Reactor
APHA:	American Public Health Association
AIPS:	Advanced Integrated Pond System
AST:	Active Sludge treatment
$BOD_{5:}$	Biological Oxygen Demand (in five days incubation)
BOKU:	The University of Natural Resources and Life Sciences, Vienna
CEPT:	Chemically Enhanced Primary Treatment
COD:	Chemical Oxygen Demand
CWs:	Constructed Wetland Systems
EC:	Electrical Conductivity
E.coli:	Enterobacter Coliform
ENPHO:	Environment and Public Health Organization
EPA:	Environment Protection Act
giZ	German Corporation for International Cooperation
HMGN:	His Majesty Government of Nepal
ICIMOD:	International Center for Integrated Mountain Development
IDA	International Development Agency
KMC:	Kathmandu Metropolitan City
MLD	Million Liter per Day
MOEST:	Ministry of Environment Science and Technology
NH_4^+:	Ammonium ion
NO_3^-:	Nitrate
RBTS	Reed Bed Treatment System
SPSS:	Statistical Package for the Social Sciences
TAR:	Total Accumulation Rate
TC:	Total Coliform
TDS:	Total Dissolved Solid
TSS:	Total Suspended Solid

TU:	Tribhuvan University
UNESCO:	United Nations Educational, Scientific and Cultural Organization
UNEP:	United Nations Environment Programme
UNICEF	United Nations Children's Fund
UNU-IAS:	United Nations University –Institute for the Advanced Study
UNWWAP	United Nations World Water Assessment Program
UN-Habitat	United Nations agency for human settlements and sustainable urban development
WHO:	World Health Organization
WQT:	Water Quality Test
WWTP:	Waste Water Treatment Plant

CHAPTER I: INTRODUCTION

The excessive use of chemicals in industries, agriculture and households, the discharged wastewater into receiving water bodies possesses detrimental effects in aquatic ecosystem. The rapid increase in population coupled with fast industrialization and intensive agricultural practices causes serious environmental problems, including the production and release of considerable amounts of toxic wastes into the water resources and soil environment (Xuliang *et al.,* 2007).

Water resources have become the site for dumping of solid wastes in their banks and draining the sewerage and effluent water in their streams. Agricultural runoff and industrial discharge without pretreatment contribute to the detrimental effects on water quality, not to mention public and environmental health.

1.1 Background

The conventional wastewater treatment methods like methods used for treatment of contaminated soils and water, namely chemical, physical, and microbiological methods are costly to install and operate. Some of the advanced wastewater treatment (active sludge treatment, microbial treatment, chemically enhanced primary treatment - CEPT, advanced integrated pond system - AIPS, stabilization ponds, aerated and non-aerated lagoons, etc.) methods are found superior than the conventional physical, chemical and microbiological processes but require sound technical knowledge, continuous monitoring high investment costs.

Phytoremediation is regarded as the popular wastewater treatment systems that have been applied in constructed wetland (CW) systems. Phytoremediation utilizes plants to decontaminate soil, water and air environment and is very cost effective and treatment process occurs in natural way with minimum technical assistance during selection of the plant variety to be used and its way of plantation during the preliminary phase (Prasad, 2003; Salt, Smith, and Raskin, 1998; Chaney *et al.,* 1997). Many researchers have reported that denitrification is the major mechanism for N removal from CW (Hammer and Knight, 1994; Brix, 1994; Oostrom. V. and Russell, 1994; Gale *et al.,* 1993; Gersberg *et al.,* 1984; Nichols, 1983; De Busk *et al.,* 1983).

1.1.1 Wastewater

The sources of wastewater vary from residential area, industrial activities, garbage pollution, commercial activities, and intense agricultural techniques to all modernized but not eco-friendly methods. Industrial effluents and domestic sewage contribute large quantities of nutrients and toxic substances that have a number of adverse effects on the water bodies and the biota that is the animal and plant life of a particular region or habitat. It was estimated that about 90% of wastewater in developing countries is still discharged directly into rivers and streams without any treatment or after retention period of sometime in stabilization ponds (Shu *et al*., 2005).

1.1.2 Wastewater Characteristics

Wastewater is generally used water, which is usually characterized by a gray color, musty odor, and solids content of about 0.1%. Chemically, wastewater is composed of organic and inorganic compounds as well as various gases. Organic components may consist of carbohydrates, proteins, fats, greases, surfactants, oils, pesticides, phenols, etc., inorganic components may consist of heavy metals, nitrogen, phosphorus, sulfur, chlorides etc. (Junsheng *et. al.*, 2010).

In domestic wastewater, the organic and inorganic portions are approximately 50% for each category. However, since wastewater contains a higher portion of dissolved solids than suspended, about 85% to90% of the total inorganic component is dissolved. Gases commonly dissolved in wastewater are hydrogen sulfide, methane, ammonia, oxygen, carbon dioxide, and nitrogen. The first three gases result from the decomposition of organic matter present in the wastewater (Chen H.M., and Lo S.L., 2009).

Biologically, wastewater contains various microorganisms that are classified as protista, plants, and animals. In a biological treatment process, these compounds can upset a treatment process or even kill the biological community and make the process ineffective (Liu *et al.,* 2000).

1.2 Wastewater Treatment System in Nepal

The oldest sewers in the core area of Kathmandu, Lalitpur and Bhaktapur were built during the Malla period for conveyance of surface drainage and domestic sewage.

Wastewater, critically exaggerated as a result of anthropogenic influence was noted to have increased significantly in Nepal since 1970, especially in the urban areas because of high growth rate of urban population, disorganized expansion of infrastructure and services for water supply, sanitation and wastewater management (Shukla *et al*., 2012).Only about 12% households of the urban centers are having access of sewer networks. As most of the pipelines are directly connected to a water body or river, only 5% of generated wastewater is appropriately being treated (WaterAid, 2008).

Common waste management practice in Nepal involves discharging of untreated sewage, domestic waste, industrial waste and municipal waste into aquatic environments without proper treatment (Jha *et al*., 2011).Government of Nepal has recognized the problem and established various treatment plants at well-designed locations of the river reach, the treatments are unable to function and the sewage mixes directly into the rivers (UNU-IAS, 2015).Wastewater treatment systems (Table 1.1) in Nepal are mainly concentrated towards the urban cities where population is high and hence the volume of wastewater produced is high which are finally discharged into the major river systems.

Due to the failure of the large treatment plants, small and decentralized treatment systems such as constructed wetlands are in high demand. Environment and Public Health Organization (ENPHO) introduced the use of constructed wetlands for wastewater treatment in Nepal as an alternative to conventional wastewater treatment technologies. The first ENPHO-designed constructed wetland system with a two staged sub-surface flow was for Dhulikhel Hospital (Shrestha, 1999).

Table 1.1: Wastewater Treatment Plants in Kathmandu Valley

(Adapted from Water and Urban Initiative Working paper Series, No. 3, April 2015)

Treatment Plant	Capacity (MLD) and Type	Status
Dhobighat: It receives wastewater from the main urban area of KMC. Constructed in 1978 with IDA funding	(15.4MLD) Oxidation pond consisting of two primary anaerobic ponds, one facultative pond, one tertiary aerobic pond. Wastewater requires pumping from Sundarighat pump station	Not operational. Out of operation almost since construction. Problem began with pumping wastewater and conveying through under water sewer

Treatment Plant	Capacity (MLD) and Type	Status
Kodku: It receives wastewater by gravity from eastern core area of Lalitpur. Constructed 1978 with IDA funding	(1.1 MLD) Oxidation pond consists of two primary/ anaerobic ponds, one secondary/ facultative pond and one tertiary/ maturation pond	Partially operational but inefficient poor operation and management: sludge accumulation and non-functioning flow control valve, resulting flow short- circulating (less detention time). Farmers tap raw sewage flowing through the sewers for irrigation
Sallaghari: It receives water from some parts of Bhaktapur urban area. Constructed in 1983 with giZ support	(2.0 MLD) Originally designed as an aerated lagoon system using diffused aeration equipment. The plant is now converted to non aerated lagoon	Partially operational. Difficulties related to pumping and operation of mechanical aerators. Farmers tap raw sewage flowing through the sewers for irrigation
Hanumanghat: It serves only a small part of the core area of Bhaktapur. Constructed in 1977 with GIZ support	(0.5 MLD) Originally developed as an aerated lagoon	Partially operational as an oxidation pond/ non aerated lagoon with low efficiency
Guheshwori: It was constructed by high power committee in 1999	(17.3 MLD) Active sludge oxidation ditch	In operation. High operating costs: in 2005, it was over 10 million (about 65% of this was for electricity). Foaming in aeration problem in aeration tank is the major technical difficulty. There is also a sludge rise/floatation problem in the secondary clarifier (Shah 2006)
Teku: It was constructed by Kathmandu Municipal Corporation	Constructed wetland- vertical flow bed	For treating seepage (from septic tank).Not in operation
Madhyapur Thimi: It was constructed with technical support from ENPHO as a pilot demonstration activity of ADB, UN-Habitat and Water Aid Nepal	Reed bed treatment system. Horizontal/ vertical flow bed	In operation. Serves around 200 households, and receives about $20m^3$/day of sewage. The municipality looks after operation and management

1.3 Constructed wetlands

Constructed wetland (CW) is a biological wastewater treatment technology designed to mimic processes found in natural ecosystems where wetland plants and their associated microorganisms remove pollutants from wastewater. Generally, the system consists of a feeding tank, reed Beds (Vertical and/or horizontal) or constructed wetland. The plants assist in the cleaning process by transporting oxygen to the microorganisms in the bed through root hairs and taking up some nutrients and other substances. As these systems are simple, effective and low cost, they are appropriate for developing countries like Nepal. They are manmade wetland system built for the purpose of wastewater as well as drainage / storm water treatments to maintain the ecosystem unperturbed. The most common type of constructed wetland system used in Nepal is the sub-surface flow system, which is also known as the Reed Bed Treatment System (RBTS). The basic features of RBTs include a bed of uniformly graded sand or gravel with plants such as reeds growing on it. The most common type of plant used in Nepal is *Phragmites karka*.

1.3.1 Applicable Reeds

The other popular reeds applied in constructed wetlands are cattails (*typha spp.*), sedges, water hyacinth (*eichhornia crassipies*), pontederia. The buckbeanses (*menyant trifolia*) and pendant grass (*arctophila fulva*) are also useful for metals uptake. The operating experiences generally show a high rate of efficiency in the removal of organic content (BOD and COD), nitrogen (TKN, NH_4^+ and NO_3^-), total suspended solids (TSS) and pathogens (E. coli, FC, TC).

Many researchers have used different plant species like Water Hyacinth *(Eichhornia crassipes (Mart.) Solms)*, Water Lettuce (*Pistia stratiotes L.)*, Duckweed *(Water Lemna)*, Bulrush *(Typha)*, Vetiver Grass (*Chrysopogon zizanioides)* and common reed *(Phragmites Australis)* for the treatment of wastewater. They have used these species for different types of contaminated waters, effluents etc.

- **Vetiver (*Chrysopogon zizanioides*)**: Vetiver grass, *Chrysopogon zizanioides* (L.) Robertysyn. *Vetiveria zizanioides* (L.) Nash, belongs to the Poaceae family; subfamily of panicoideae, tribe andropogonae and subtribe sorghina, and the genus includes ten species (Bertea and Camusso, 2002). Vetiver grass was first recognized in 1995 in the Indian sub-continent and is common to flood plains and stream banks, but can also be found throughout the tropical and subtropical regions of Africa, Asia, America, Australia, and Mediterranean Europe (Maffei, 2002). Another special characteristic of Vetiver is the high tolerance of a range of extreme soil conditions, especially heavy metal contamination and has potential to rehabilitate contaminated water and soil. It has been recently introduced in Nepal for its various applications for riverbank protection, soil erosion control, landfill leachate as well as organic wastewater treatment. It has wide application in the field of soil and water conservation; land rehabilitation, agricultural improvement, disaster mitigation, handicraft, pollution control, water quality improvement and many other environmental applications, particularly the looming food crisis in many parts of the developing world.

- **Common reed (*Phragmites karka*)**: *Phragmites sp.* is a cosmopolitan genus of about 4 species, of which 3 occur in tropical Africa. The species are very similar, distinguishing characters overlap and combinations of characters are needed to distinguish the species. The genus is sometimes considered monospecific. Popular as common reed and ecocrop, common reed (*Phragmites karka*) is also used in decontamination of wastewater among its various application such as soil stablization in the shores of lakes and streams, wastewater treatment in the constructed wetlands, roots are used to heal broken bones and rheumatic pains and also used in curing diabetes since centuries ago.

Vetiver and common reed are the two widely used macrophytes in Wastewater Treatment. Though belonging to the same family i.e. Gramineae and being used for similar purposes, they both have distinct morphological and distribution features. *Phragmites karka* like other *phragmites sp.* has comparatively shallow root system and has rhizome or seeds from which the new shoot grows. Their growth is very slow after once harvested. Comparatively Vetiver grows by vegetative propagation so has faster rate of growth and nutrient absorption. *Phragmites sp.* has become weedy pest in many wetlands and waterways around the world but due to sterile seeds, no rhizomes and vegetative propagation of Vetiver, it has no threat to weed potential. Their wastewater treatment efficiency also varies widely depending on their structural parameter and root growth as the treatment done by plants or the nutrient uptake from wastewater is the function of their roots and biomass production.

1.3.2 Design Characteristics and Pollutants Removal

The major concern in the design of surface flow constructed wetland includes hydraulic and hydrological conditions; BOD and TSS removal mechanisms; nitrogen removal efficiency; vegetation selection and management; construction details, etc. Various physical (sedimentation, filtration), chemical (adsorption, exchange) and microbial (aerobic and anaerobic degradation, microbial mediated redox, etc.) processes have been occurred for the removal of pollutants (Table 1.2) like soluble organics, phosphorous, nitrogen, metals and pathogens too.

Table 1.2: Wastewater Purification Mechanism in Constructed Wetland

Wastewater Parameters	Removal Mechanism
Suspended solids	Sedimentation / Filtration
Soluble organics	Aerobic / Anaerobic microbial degradation
Phosphorous	Matrix sorption & Plant uptake
Nitrogen	Ammonification followed by nitrification; Denitrification; Plant uptake; Matrix absorption; Ammonia volatilization
Metals	Adsorption and cation exchange; Complexation; Precipitation; Plant uptake; Microbial mediated redox reactions
Pathogens	Microbial excretion antibiotics from plants and UV irradiation

(Cooper *et al.*, 1996)

ENPHO, a non-governmental organization has introduced Constructed Wetland technology as a low cost, decentralized and effective option for wastewater treatment and recycling in Nepal (Table 1.3). In 1997, the first CW was built at Dhulikhel Hospital under the design and technical supervision of Nepali and Austrian researchers from University of natural resources and Applied Life Science (BOKU), Vienna, Austria. Following the successful demonstration of CW technology in the Dhulikhel Hospital, this technology has been replicated at several places.

Table 1.3: Constructed Wetlands in Nepal

S. N.	Location	Type of Wastewater	Treatment Capacity
1	Dhobighat	Domestic	15.4 m^3/day (not operational)
2	Dhulikhel Hospital	Hospital waste	Designed for 10m^3/day but treating 40m^3/day
3	Private house at Dallu	Grey water	0.5m^3/day
4	Kathmandu metropolitancity	Septage	40m^3/day
5	Kodku	Domestic	1.1 m^3/day (partially operational)
6	Hetauda Industrial Estate	Industrial	16.4 m^3/day (partially operational)
7	Malpi international school	Institutional	25m^3/day
8	Sushma Koirala memorial plastic and reconstructive surgery hospital	Hospital waste	15m^3/day
9	Kathmandu University	Institutional	40m^3/day

S. N.	Location	Type of Wastewater	Treatment Capacity
10	Staff quarter of Middle Marsyangdi hydro electric power station	Domestic	$26m^3$/day
11	ENPHO laboratory	Domestic and laboratory	$1m^3$/day
12	Pokhara Municipality Reed Bed (Constructed Wetland)	Landfill leachate	<0.115 m^3/day
13	Kapan monastery	Institutional	$17m^3$/day
14	Septage and landfill leachate treatment plant	Septage and landfill leachate	Septage: $75m^3$ Leachate: $40m^3$
15	Sunga	Municipal	$25m^3$/day
16	Tansen Municipality Reed Bed (Constructed Wetland)	Municipal	< 0.030 m^3/day

Source: Nyachhyon (2006), Decentralized wastewater management using constructed wetlands in Nepal, 2008

1.4 Phytoremediation

Phytotechnologies an emerging technique during the last two decades and plant based bioremediation technologies that have been collectively termed as phytoremediation. Phytoremediation (phyto: plant and remediation: correct evil) is a set of technologies that use plants to clean contaminated sites and is relatively new one. Phytoremediation comes from a variety of research areas including constructed wetlands, oil spills and agricultural plant accumulation of heavy metals. The idea of using metal accumulating plants to remove heavy metals and other compounds were first introduced in 1983 (Singha *et al.,* 2012), but the concept has actually implemented for the last 300 years (Henry, 2000). Phytoremediation has also been called green remediation, botano-remediation, agro remediation and vegetative remediation (Erakhrumen, 2007).

1.4.1 Reclamation Techniques of Phytoremediation

Heavy metals can be removed from the contaminated subjects either by phytoextraction, rhizofiltration or by phytostabilization (EPA/600/R-99/107, Figure 1.1). In most of the cases the metal can be uptake by the plants through roots and thereby translocated within the plant. Finally, the toxic metal can be either disposed or extracted from the harvested product. Nanda Kumar *et al.* (1995) reported that the concentration of lead up to 500mg/l was phytotoxic to *Brassica juncea*.

Phytoremediation is a general term for several ways in which plants are used to remediate sites by removing pollutants from soil and water (Table 1.4). Plants can degrade organic pollutants or contain and stabilize metal contaminants by acting as filters or traps.

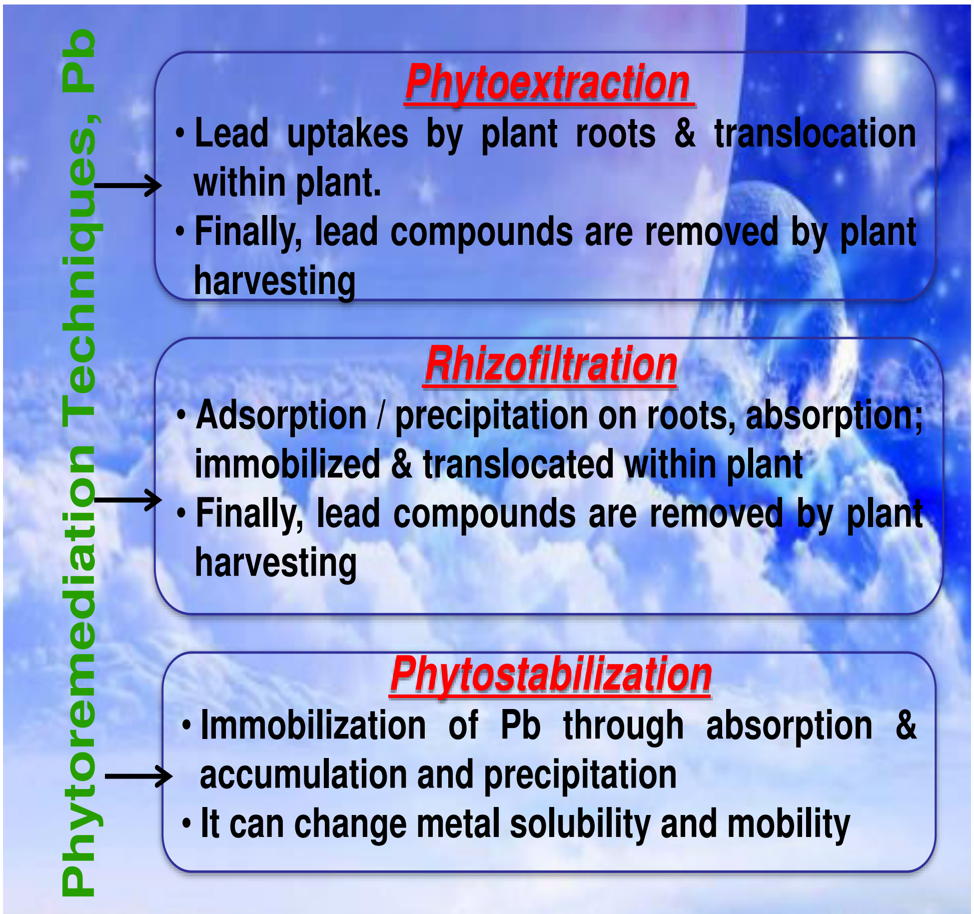

Figure 1.1: Example of Phytoremediation of Lead

Table 1.4: Fundamental Phytoremediation Mechanism

	Phytoremediation Mechanism	Brief Description
1.	Phytotransformation	Uptake of contaminants from soil & groundwater by plants and their subsequent transformation in roots stems & leaves.
2.	Rhizosphere Bioremediation	It occurs in the root-zone that results in increase of soil organic carbon, and bacterial and fungal populations.
3.	Phytostabilization	It refers to holding of contaminated soils in place by vegetation, and physical or chemical immobilization of contaminants
4.	Phytoextraction	Use of metal accumulating plants that translocate metals from the soil to their roots and concentrate the metals to aboveground stems and leaves.
5.	Rhizofiltration	Adsorption of metal contaminants from surface waters (Wetlands treatment) or groundwater

Yan-de et al., 2007, EPA 542-B-99-003

1.5 Statement of Problem

As it is clearly visible that with the increasing urbanization and development, the major rivers and their tributaries are becoming the dumping sites of all kinds of domestic, industrial, medical, construction and other various kinds of waste, the river systems which are the center of our traditional values are being deteriorated day by day. Organic, medical, industrial and chemical pollution constitute the third major problem after land degradation and urban sanitation, which are first and second, respectively (Zinabu and Zerihun, 2002).

Some of the best methods include Active Sludge treatment, Microbial treatment, Chemically Enhanced Primary Treatment (CEPT), Advanced Integrated Pond System (AIPS), Ozone bioreactor, UV radiaton treatment, Stablization ponds, Aerated and non aerated Lagoon system which are very costly and require good technical knowledge on handling the process. These modern and sophisticated technologies are not always feasible in country like Nepal. The Bagmati river and their tributaries around Kathmandu Valley which are loosing their originality and suffering for their stable existence due to contamination by different kinds of anthropogenic sources for which wastewater disposal from houses, schools/colleges, hospitals, industries etc. are the major cause. For this reason, the treatment of wastewater is not only desirable but also necessary.

1.6 Justification of Study

The main focus of my study was to compare the rate of growth and wastewater treatment potential of *Chrysopogon zizanioides* (vetiver) and *Phragmites karka* (common reed) plants as the wastewater treatment potential and the rate of growth of plants are directly interrelated and play vital role in the selection of the better option in order to find out the best and easy solution to water pollution problem at a very minimum cost of money and time.

Wastewater treatment by phytoremediation is natural and widely used technology in most of the countries around the world. In Nepal, in the recent years application of community level wastewater treatment using *Phragmites karka* is in practice but research using some other varieties which could give similar or even better performance in wastewater treatment is still lacking. *Chrysopogon zizanioides*has been started for the various purpose of slope stablization and riverbank protection, lecheate treatment, degraded land reclamation but very least studies have been done for its importance and application in wastewater treatment. This experimental research would be helpful for bringing out a better solution to the present scenario of river pollution.

1.7 Research Question

The objectives and methods were set to find out the answer to following research questions.

- Can Vetiver and common reed both survive and develop in the same soil type and wastewater concentration of study plot?
- To what percent will the physical, chemical and microbial concentration change in the different treatment ponds?
- What are the changes in soil nutrients concentration after wastewater treatment and what is their relation to the morphological change in plants and change in test parameters of wastewater?

1.8 Objectives of Study

The broad objective of the study was to determine the wastewater treatment efficiency of Vetiver (*Chrysopogon zizanioides*) and common reed (*Phragmites karka*) in constructed wetland system.

The specific study objectives were as follows:

- To study the morphological changes (height, lateral growth, leaf colour, decay and new growth) in the Vetiver and common reed at weekly interval
- To determine physical (pH, temperature, conductivity) chemical (biochemical oxygen demand, chemical oxygen demand, nitrate-N, Phosphorus, chloride, carbondioxide) and microbial (total coliform) parameters of wastewater before and after treatments at an interval of two weeks after three month of plantation
- To analyze soil nutrient change (percent organic matter, percent organic carbon, percent total nitrogen and available phosphorus) in relation to change in morphology of plants and change in chemical concentration of water

1.9 Limitation of Study

- The research was conducted in very small scale on experimental basis.
- The research duration was only for six month from March to August.
- The constraints due to mixing of rainwater from the drainage outlets system from the building and the ground water flow during monsoon were not considered.

1.10 Organization of Study

Chapter I of this study introduced the problem statement and described the specific problem addressed in the study as well as design components. Chapter II presents a review of literature and relevant research associated with the problem addressed in this study. Chapter III presents the methodology and procedures used for data collection and analysis. Chapter IV contains an analysis of the data and presentation of the results. Chapter V offers a summary and discussion of the researcher's findings, conclusion and recommendations for future research.

CHAPTER II: LITERATURE REVIEW

The freshwater bodies have become polluted by nutrients originating from agricultural, domestic and industrial sources. This situation causes these freshwater bodies to become nutrient enrichment as the wastewater and sewage from domestic sources, industrial sources and storm water directly from the roads and streets in the urban areas and the runoff originating from the agricultural lands are directly drained into the rivers and other water bodies. In South Asia only about 63% of the rural population, which counts, about 778 million people use open defecation (UNICEF / WHO, 2008). United Nations World Water Assessment Program (UNWWAP) estimated that the two million tons of sewerage and agricultural waste was discharged everyday into World's river water. According to this report, the amount of wastewater produced annually was about 15,000Km^3 (UN WWAP, 2003).

The effects of industrial activities on the environment in the country are becoming evident through the pollution of water bodies and human habitat in major cities, rivers and lakes (Dierig, 1999; Zinabu and Zerihun, 2002).

2.1 Cause of Water Pollution

The misuse of water resources and poor water management practices have resulted in depleted supplies, falling water tables, shrinking inland lakes and stream flows diminished to ecologically unsustainable levels.

Nina (2013) in her research found that the use of chemical fertilizers and over exploitation of local forest resources threatens the sustainability of ecosystems, which urges for alternative solutions.

Khan (2005) reported that the components of inorganic pollutants are heavy metals, such as lead, arsenic, cadmium, copper, zinc, nickel, and mercury, which were continuously added into the environment via disposal of urban sewage sludge and industrial wastes in agricultural soils and via agrochemical usage.

Water quality deterioration can be attributed to pollution entering surface and groundwater from sources such as runoff, municipal and industrial discharges (Hanping *et al.*, 2004 and Truong, 2000).

Water pollution is originating mostly from human activity and occurring even more frequently, decreasing the amount of water suitable for many uses (Gustard *et al.*, 2002).

2.2 Water Pollution in Nepal

River pollution has become an integrated environmental problem for Nepal. The problem was more pressing in urban areas of Kathmandu Valley due to increasing concentration of population.

Shukla *et al*. (2012) studied on industrial wastewater entailed that the industries like brewery, distillery, cigarette, tobacco, cement, iron, steel, rosin, turpentine, soap, oil, ghee, jute, paper, sugar and leather had been producing significant amount of wastewater in the country industries. Total of 4500 industrial units of different sizes were estimated to be operating in different parts of the country and the concentration of industries are large in Kathmandu Valley and some urban centers (Birgunj, Biratnagar, Bharatpur, Butwal and Bhairahawa) in Terai- Madhesh.

Jha *et al*. (2011) reported that the wastewater generated in most industries mixed with the municipal sewerage system while the solid industrial waste was collected and dumped into pits or in open spaces.

ICIMOD, MoSTE/ GoN, UNEP (2007) entailed that only about 40% of the population has access to sewer facility within 232km long sewer system developed in Kathmandu Valley. The Bagmati River and its tributaries have been an integral part of the Kathmandu Valley civilization. The rivers not only became a source of sustenance for the Valley's population but also gained religio-cultural significance.

2.3 Water Resource Management in Nepal

Several government and non-government institutions, civil societies and other stakeholders are working for environmental Water Resources Strategy endorsed by the Government of Nepal in 2002 envisions integrated approach to water resources development wherein exploring the possibility of wastewater recycling/use has been identified as one of the alternatives to approaching/enhancing water security, at least in the areas that are known to facing scarcity of water. The water quality standards for safe use of wastewater in agriculture, aquaculture, livestock watering, recreation and environmental uses, published in the Gazette of the Government of Nepal in 2008 enforces the national commitment to promote safe use of wastewater. Contrarily, the emphasis in translating the policy emphasis into actual plans and programs for safe wastewater use has been largely lacking in most water sector development agencies and also those concerned with the health and environmental issues (WECS, 2011)

2.4 Policy responses on water resource conservation

Efforts to conserve water resources undertaken by the government through legal measures are summarized in the acts and regulations given below.

- Environmental Protection Act (EPA) (1996) and Environmental Protection Rules (EPR) (1997) and its Amendment (1999)
- Water Resources Act (1992), Water Resources Regulations (1993)
- Solid Waste Act (1987), Solid Waste Regulations (1989)
- Electricity Act (1992)
- Soil and Watershed Conservation Act (1982)
- The Aquatic Life Protection Act (1961), Aquatic Animals Protection Act (1965)
- Patent, Design and Trademark Act (1965)

Nepal is a party to a number of broader international conventions and treaties, including the Rio Conference of 1992 related to water, environment, and development (National Policies of Nepal Government, 2011).

- Water rights

Upstream communities have begun to demand the right to control the use of water from streams originating or flowing through their own areas also called as Riparian rights.

(Nepal: State of the Environment 2001, SoE Report Nepal)

2.5 Researches on Water Quality of Bagmati River

The physicochemical and biological parameters of Bagmati River have been studied by many researchers namely Gautam *et al.* (2013), Khanal and Dahal (2011), Shrestha (2009), Chhetri (2006), Sharma *et al.*(2005), ENPHO (2003), Aratha (2003), Yadav (2002) and many more. Their reports showed that the water quality of Bagmati River had been degrading day by day and the pollution level increased as the city passes by the city core. Gautam *et al.* (2013), NWCF (2009) and ICIMOD (2007) also reported that the physico-chemical quality of the Bagmati River and its tributaries had decreased as the river approached to the urban sector.

2.6 Studies on Constructed Wetlands and Reeds

There has been developed various forms of wastewater treatment systems varying from simple stablization pond to the most technically complex and costly systems like chemical pulps, microbial treatment and Ozonation process. The most technically advanced treatment system can purify upto 99.99% pollutants from the toxic wastewater, which is not practicable for the technically and economically poor country like ours. Easy and natural processes like constructed wetlands and reed bed treatment systems are better solution for us as they are cost effective and rely on natural capacity of plants on absorbing nutrients and toxic chemicals from wastewater. Selection of appropriate plant is the major concern in this technique.

Asghar *et al.* (2013) performed experiments on wetland using *Cyperus alternifolius* plants and shrubs. He found that the wetland was efficient for COD removal. Khateeb *et al.* (2013) study recommended that subsurface flow constructed wetland is useful for treatment of sewage water. Mthembu *et al.* (2013) found that wetland treatment of wastewater is economical. He suggested constructed wetland could be an alternative wastewater treatment technology.

Gurung, A. and Oh, S.E. (2012) studied about the constructed wetlands in Nepal. Relatively higher pollutant removal efficiency (>95%) in terms of suspended solids, organic pollutants and ammonium ion (NH_4^+-N) were found in all the existing CWs. Despite having higher removal rate of organic pollutants, CW technology is still in its infancy stage in Nepal. Therefore, further research and development is necessary for making the CW technology as a promising decentralized technology for treating wastewaters in Nepal. Dong *et al.* (2012) used aquatic plants in wetland for waste treatment and found it efficient for reducing COD.

Badejo *et al*. (2011) studied the prototype reed bed using Nigerian species (*Vetiver*ia *nigritana*) and Common reed (*Phragmites karka*) and the result showed reduction of BOD 82.0% and 85.0%, TDS 72.0% and 73.0%, PO_4^{-3} 78.0% and 81.0%, NO_3^- 61.0% and 65.0% for *V. nigritana and P. karka* respectively.

Kayranli *et al.* (2009) studied the performance of newly constructed wetlands for one year in Glaslough and five years in Dunhill for matured integrated constructed wetland. Shrestha, D. Maharjan, S. (2009) introduced low-cost natural treatment options like CWs and the related RBTs to mitigate the problem of water pollution in Nepal in several places like hospitals, universities and other institutions, and as community systems. The plant species *Phragmites karka* was used in this process. Compared to other large and expensive technologies, CW and RBT require less land and are less expensive for construction, operation and maintenance. Kadlec and Wallace (2009) pointed out that CWs with sub-surface technology were started in North America during the early 1970s. Since the mid 1980s, the concept of using constructed wetlands has gained increasing support in Southern Africa. At present, CWs are in operation, in Asian countries like India, China, Korea, Taiwan, Japan, Nepal, Malaysia and Thailand for various types of waste wastewater.

Chen *et al*. (2008) applied municipal wastewater to constructed wetland vegetated with common reed (*Phragmites australis*), water bamboo (*Zizania aquatica*) and cattail (*Typha latifolia*). The system showed excellent results in removing BOD_5, COD, TSS, TP and NH_3-N. Singh *et al.* (2008) summarized the performance of the DEWATS from July 2006 to August 2007 in the removal efficiencies of TSS, BOD5, COD, NH4–N, TP and FC. The ABR was found to be very effective in the removal of organic pollutants and could achieve TSS removal up to 91%, BOD5 up to 78% and COD up to 77%. The average removal efficiencies of the DEWATS was 96% TSS, 90% BOD5, 90% COD, 70% NH_4- N, 26% TP and 98% FC.

Singh *et al.* (2007) had carried out monitoring of the performance of Sunga wastewater treatment system over its first year of operation shows that it removed organic pollutants highly efficiently (up to 98%: TSS, 97%: 96%: BOD_5 and 97%: COD). It was also found that the ABR was very effective in removing organic pollutants and could remove up to 74% of TSS, 50% of BOD_5 and 18% of COD.

Chomchalow (2006) comparative study of Vetiver grown in domestic wastewater from the Royal Irrigation Department community revealed that different ecotypes exhibited different growth and adaptability. 'Surat Thani' ecotype was found to exhibit the highest ability to reduce nitrate (49.33%), bicarbonate (42.66%), EC (5.81%), and TSS (82.78%), while 'Monto' cultivar exhibited the highest ability to reduce BOD (75.28%), total nitrogen (92.48%), potassium (14%) and sodium (3.14%). The efficiency of wastewater treatment was found to increase with the age of Vetiver plant, and the highest was at three months of age.

Klomjek and Nitisoravut (2005) conducted research on artificial wetlands for removal of pollutants from saline wastewater using cattail (*Typha angustifolia*) and Asia crabgrass (*Digitaria bicornis*). The wetland vegetated with these plants was successful in reducing contamination level of BOD_5, NH_3-N, TP and Suspended Solids.

Bista, K.R. and Khatiwada, N.R. (2004) has carried out study on two existing operating treatment plants (KU and DH), which have operated for 1 and 5 years. They highlighted about the popular local reeds (*Phragmites Karka spp*) in subsurface horizontal bed and vertical bed systems. The organic matter removal performance was varied from 86% to 93 %, consisting the effluent COD concentrations of 20 to 38 mg/L depending on organic loading rate and age of wetland. Also nutrient removal efficiency was satisfactory. The removal performance of faecal coliform was about 98.3 %.

Njau and Mlay (2003) used Vetiver and common reed *(Phragmites maritianus)* (a species similar to *phragmites karka*) to treat domestic type of wastewater from the University main Campus of Dar es Salaam and found that Vetiver grass performed better than *Phragmites mauritianus* in removing of pollutants. For instance It was found from the treatment plant that: the organic removal (BOD) was on average 61.85% and 67.47% and COD of 37.9% and 46.2% for Vetiver and *Phragmites mauritianus* grass respectively. Truong (2003) applied Vetiver in an application of removal of nitrogen and phosphorous at a nutrient rich sites where it could remove up to740 kg N $ha^{-1}$and 110 kg P ha^{-1}over three months at a nutrient-rich site (Vieritz *et al.*, 2003) which was much more than Rhodes grass, Kikuyu grass, Green Panic, Forage Sorghum, Rye grass, and Eucalyptus trees. Wagner *et al.* (2003) in an experiment to determine the upper tolerance limit of Vetiver (*Chrosopogon zizanioides* or *Vetiveria zizanioides*) to N and P applications, showed that Vetiver growth increased with the level of N supply up to 6000 kg $ha^{-1}year^{-1}$. However, very little growth response occurred at rates higher than 6000 kg $ha^{-1}year^{-1}$; although rates up to 10,000 kg N $ha^{-1}$of N did not adversely affect Vetiver growth. Similarly, no growth response occurred at P rates higher than 250 kg ha^{-1} $year^{-1}$. However, its growth was not adversely affected at P application rates up to 1000 kg $ha^{-1}year^{-1}$. Xia *et al.* (2003) conducted study over three years which showed that Vetiver could tolerate a time of submergence (more than 120 days) much higher than the other plants included in the study, i.e. Bahia Grass: 60 - 70 days, Carpet Grass: 32 - 40 days, Sour Paspalum: 25 - 32 days, St. Augustine: 18 - 32 days, Centipede Grass: only 7 - 10 days. Truong and Smeal (2003) carried out the comparison among wetlands. When growing under wetland conditions, Vetiver (*Vetiveria zizanioides*) had the highest water use rate compared with other wetland plants such as *Iris pseudacorus, Typha spp.*, *Schoenoplectus validus*, *Phragmites australis*. It has been estimated that for 1 kg of dry shoot biomass, Vetiver would use 6.86 L $day^{-1}$of water.If the dry matter yield of 12-week-old, at the peak of its growth cycle, was 40.7 t ha^{-1}, a hectare of Vetiver would potentially use 279 kL $ha^{-1}day^{-1.}$

Tyrrel *et al*. (2002) studied the treatment of Leachate through sub-surface flow using clay loam soil substrate planted with grass (*Agrostis Stolonifera*).

Truong and Hart (2001) used vetiver for domestic effluent treatment for four days and the removal in total nitrogen was 94%, total P was 90%, EC by 50%.Australian research on Beelarong community farm showed that after five months of growth, the wastewater after passing through 5 rows of Vetiver had its total N reduced by 99% (from 9.3- 0.7 mg/L), total P by 85% (from 1.3-0.2mg/L) and fecal coliforms by 95% (from 500 to 23 organisms/100ml).

Cull *et al.* (2000) found that the average consumption rate of 600 ml $day^{-1}pot^{-1}$over a period of 60 days, Vetiver used 7.5 times more water than *Typha*. Grimshaw (2000) carried out the morphological studies on *Vetiveri zizanioides.* It did not produce seeds that germinate under normal field conditions. *Vetiveria nigritana* seedlings were easily controlled. Many researches on Vetiver till date make it clear that *Vetiveria zizanioides* showed its best performance compared to any other species of vetiver.

Extensive researches in over 100 countries including Australia, China, Guam, Thailand and India has demonstrated that Vetiver is tolerant of a wide range of soil pH and elevated levels of salinity, sodicity, aluminium, manganese, arsenic, cadmium, chromium, nickel, copper, mercury, lead, selenium, zinc and other various kinds of toxic chemicals which is not possible for any other plants grown under the same condition to have their 100% survival (Troung and Baker, 1998).

When Vetiver was used to treat the polluted river in central China, the removal percent of total P was 93.7% and 99% in two and four weeks and that of total N was 58% and 71% respectively (Annon, 1997; Zheng *et al.,* 1997).

2.8 Studies on Phytoremediation

Gupta, P., Roy, S. and Mahindrakar, A.B. (2012) carried out research on application of some plant species that have been applied for phytoremediation purpose. The study entailed that waste water treatment plants like Water Hyacinth *(Eichhornia crassipes)*, Water Lettuce (*Pistia stratiotes L.)*, Duckweed *(Water Lemna)*, Bulrush *(Typha)*, Vetiver Grass (*Chrysopogon zizanioides), (Vetiveria nigritana)* and Common Reed *(Phragmites Australis, Phragmites karka, Cynodon dactylon, Cyperus alternifolius*, *Agrostis Stolonifera),* Water bamboo (*Zizania aquatica*), Cattail (*Typha angustifolia*) and Asia crabgrass (*Digitaria bicornis*) were found to be suitable through phytoremediation technique.

Northwestern University (2007) applied phytoremediation for pollution control, however, has several limitations that required further research on plant and site-specific soil conditions. Phytoremediation was mainly confined to the area occupied by the root systems. In addition, non-perennial plants, particularly those with slow growth and low biomass production require a long-term commitment for remediation. Environmental conditions also determine the efficiency of phytoremediation as the survival and growth of plants are adversely affected by extreme environmental conditions, toxicity, and the general conditions of soil in contaminated lands.

Phytoremediation being part of constructed wetland treatment, is clean, simple, cost effective, non-environmentally disruptive (Wei and Zhou, 2004; Zhou and Song, 2004), and most importantly, its by-products can find a range of other uses (Truong, 2003).

Zhu *et al.* (1999) and Abd-Elmoniem, (2003): (Adel E.EL-Leboudi *et.al.* ,Egypt) Total accumulation rate (TAR) of young water hyacinth plant for roots, stem & leaves were 12.7, 11.4 and 10.5mg Pb/kg biomes/day respectively during the 5 days exposure period.

The plant accumulated up to 0.5% Ni, 0.8% Cd, 1.3% Cu, 1.3% Zn, and 5.5% Pb by weight (Zayed *et al.,* 1998; Wang *et al.,* 1996). Adiningsih *et al.* (1998) conducted a study in a greenhouse, using water hyacinth (*Eichornia crassipes*) to remediate soil polluted by lead and cadmium. The results showed that these plants grew well in contaminated soil, and were able to accumulate lead and cadmium taken up from the soil. The content of lead and cadmium in the plants (on a dry matter basis) reached as high as 400 ppm. Phytoremediation is the in situ use of plants and their associated microorganisms to degrade, contain or render harmless contaminants in soil or groundwater (Cunningham *et al.,* 1996).

Adiningsih *et al.* (1998) conducted a study in a greenhouse, using water hyacinth (*Eichornia crassipes*) to remediate soil polluted by lead and cadmium. The results showed that these plants grew well in contaminated soil, and were able to accumulate lead and cadmium taken up from the soil. The content of lead and cadmium in the plants (on a dry matter basis) reached as high as 400 ppm.

The water hyacinth is perhaps one of the most commonly cited species for phytoremediation of polluted water (Gupta 1980; McDonald and Wolverton 1980). The plant has a rapid growth rate and can hyperaccumulate nutrients (Cornwell *et al.*, 1977) as well as heavy metals (Wolverton *et al.*, 1975).The floating aquatic plant water milfoil (*Myriophyllum spicatum*), at a biomass density of 0.02 kg/L, rapidly accumulated Ni, Cd, Cu, Zn, and Pb.

CHAPTER III: MATERIALS AND METHODS

After detail study of the types of wastewater from the college and its drainage, need assessment was done in order to know the idea and concern of the local people about the wastewater treatment using plants naturally. Khwopa college ground was selected as study area as it was the origin site of wastewater which was further drained into nearby river resource along with other runoff wastewater.

3.1 Study Area

"Khwopa College", a renowned Academic institution, undertaking of Bhaktapur Municipality was selected as study site (Figure 3.1). The college was established in 2056 BS as a higher secondary school and later in 2058 BS developed as an institution where students can build their educational career from +2, to master level. This college lies in Dekocha-5, Bhaktapur covering area of 55 ropani (1.83 hectares) and serves 1787 (Bachelors and Masters) and 2039 (+2) students from fifty two different districts of Nepal and more than 300 faculties, part time and full time staffs are working in this institution (Academic Browser, 2014).

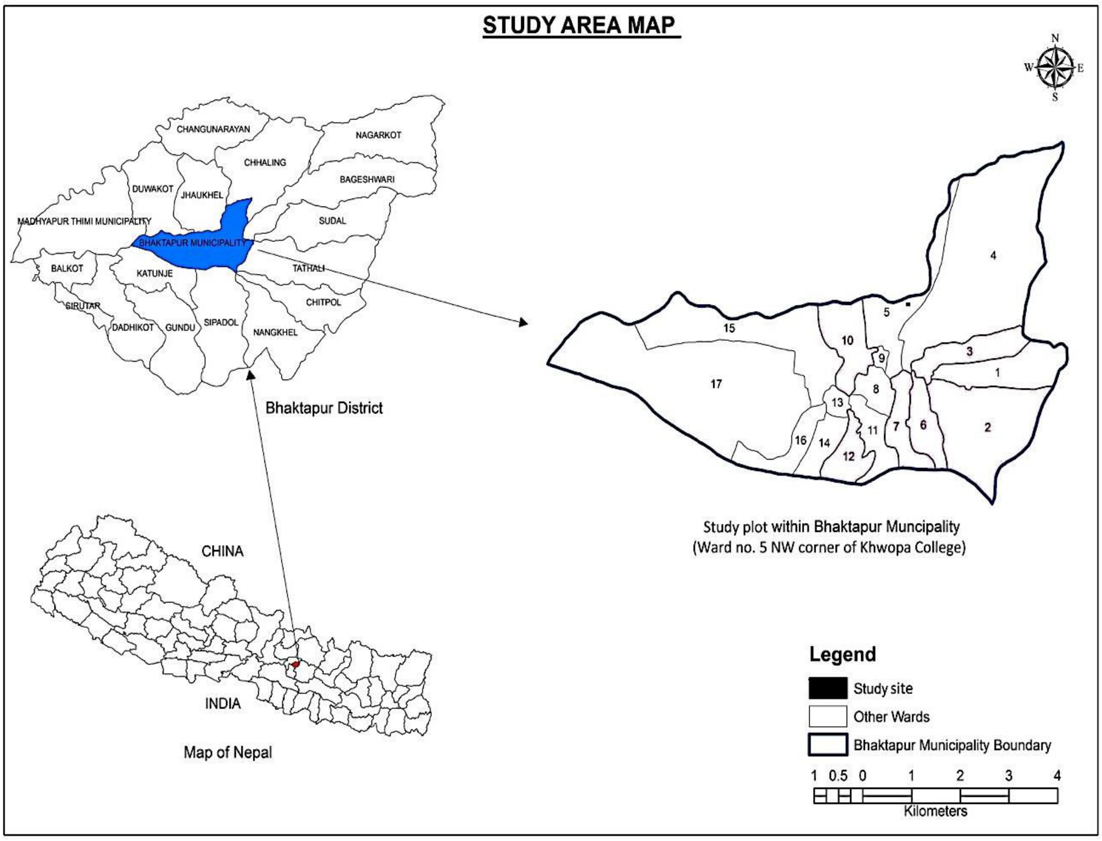

Figure 3.1: Study Area (ArcGIS 10.1 October)

Khwopa college was selected for the study as it consisted number of beneficiaries belonging to different districts and is undertaking of Bhaktapur Municipality. This research would bring applicable approach of treating the wastewater before being discharged into the river systems. This research would would be an demo model of natural way of wastewater treatment at individual level which can be promoted for its use to various hospitals, schools, offices, industries or households which are all the contributors to water pollution.

Conceptualization about Design Approach

The wastewater treatment design site was at North- West corner of the Block A of College ground in an unused boulders refilled area near to the wastewater open drainage (Figure 3.2). The location was selected based on the drainage system of wastewater and availability of adequate sunlight for the proper growth of plants.

3.1.1 Design Features

Figure 3.2: Location of the Study Site, (Google Earth Image, 17th September, 2014)

The fundamental applied design features for the study were as follows (Figure 3.4):

Greenhouse Area	:	7m x 5m
Roof Materials	:	Locally available materials (Bamboo, Common reed, plastic.
Wetland Pond Size	:	1m x 3m x (0.3m to 0.5m)
Number of Wetlands	:	4
Gradient Flow Separation	:	0.5m
Reeds	:	Common reed and Vetiver (Mixed and alternative with control)
Refill Materials	:	Brick pieces, Pebbles, Sand

The wastewater flow in each pond was maintained in such a way that the raw wastewater enter through 2 inch diameter PVC pipe into the porous pipe line through gravel and wire net assembly before the front row of each treatment pond so that the unwanted solid wastes were subjected to trap and only liquid waste encompassed to the plants roots by subsurface flow. The treated water passed down the slope through each row and finally reached to the outlets at the end of each pond (Figure 3.3). The monitoring holes were made randomly in between the rows in order to test and compare the physicochemical quality of wastewater after treatment. A narrow canal was dug around the study site in order to allow drainage of the surface drain water into other ways of outlet system to minimize the contamination error.

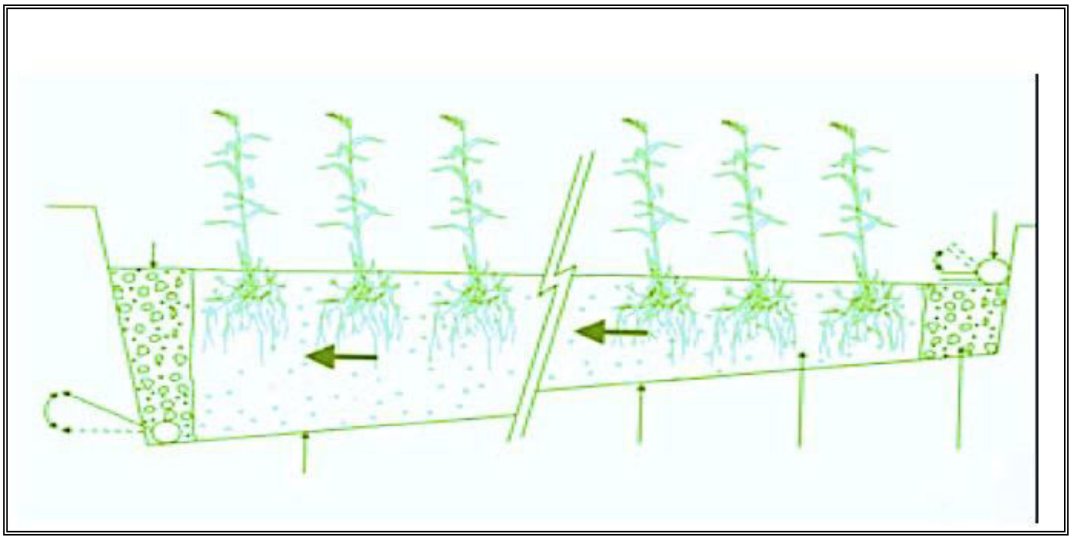

Figure 3.3: Wastewater Flow

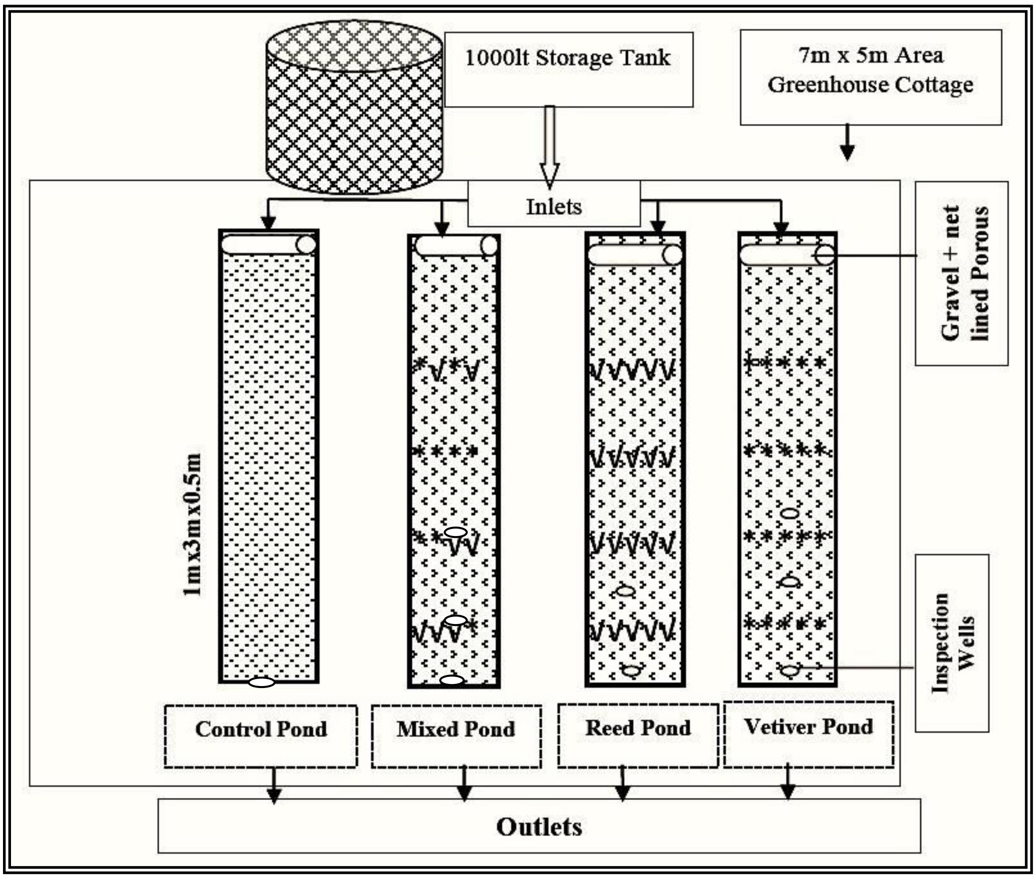

Figure 3.4: Construction Design

3.2 Research Design

The research had initiated after the management support from the college for building the prototype of construction-wet land at the college premise. The stipulated design features (Figure 3.4) were applied for the construction and the wastewater was discharged into the system and all study parameters were analyzed as per the objectives of the study. The reading of morphological change in plants and sampling for the laboratory tests was done after 3 months (after started irrigating with wastewater) at an interval of every 15 days.

Various literatures were concerned for the construction and operation of the design. The laboratory data were generated through direct sampling and analysis whose values were kept consideration for the efficiency analysis of the system. The obtained data were statistically analyzed and the report was prepared and presented.

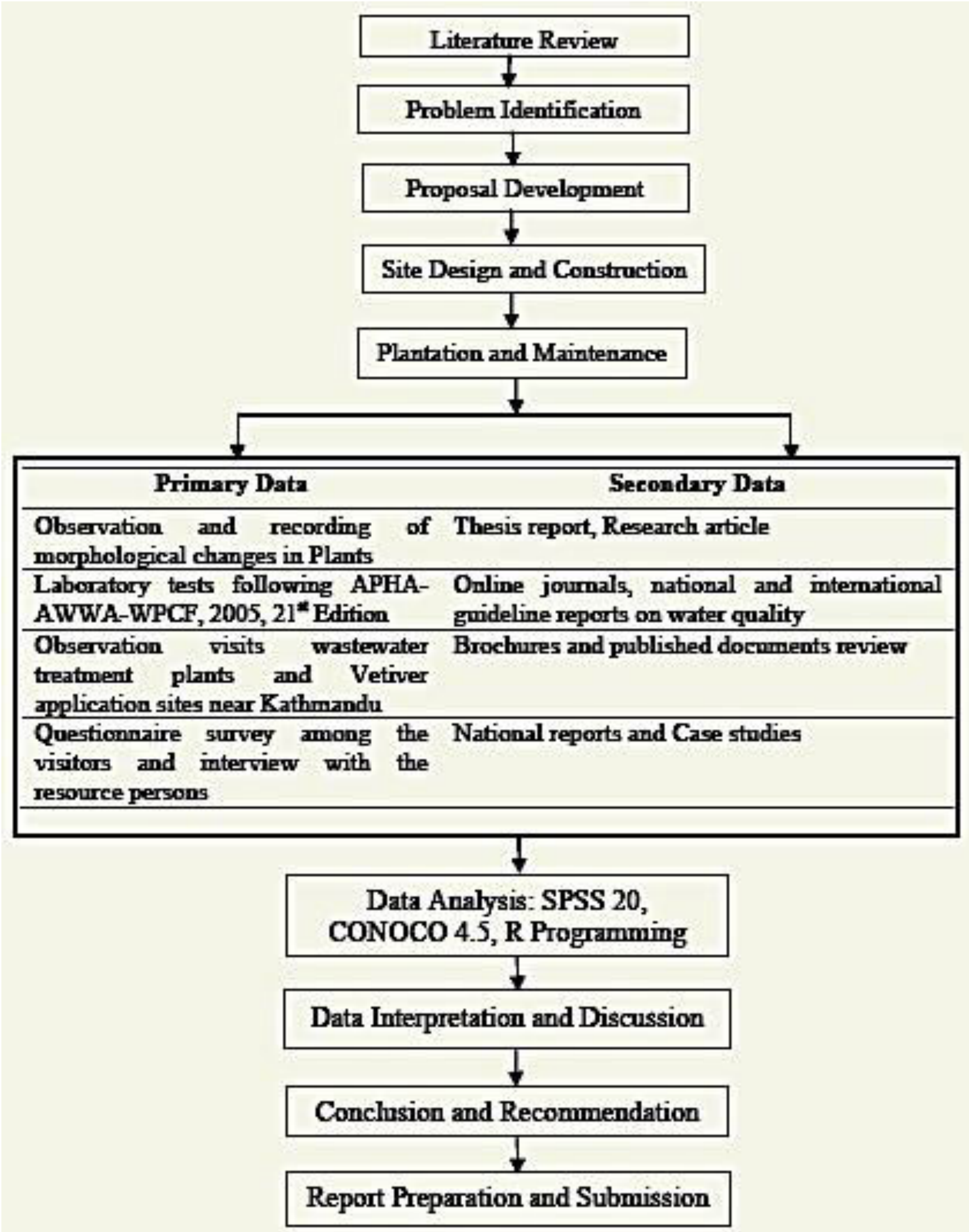

Figure 3.5: Study Design

3.3 Study Variables

The general morphological parameters like growth height, number of vegetative leaves, etc., were considered. Likewise, influent and effluent wastewater quality parameters like pH, Electronic conductivity, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrate- Nitrogen, Total Phosphorus, Chloride, Free Carbon dioxide and Coliform were analyzed and utilized for the efficiency of the prototype of the applied design.

3.4.1 Sampling Points and Frequency

The samples for the water quality tests were collected from the four monitoring wells or the holes prepared in such a way that make comparison between the outlets for the same variety of plant planted in different ways individually or mixed can be done by calculating their values. The water samples from each pond were taken at an interval of every fifteen days for six times after three months of plantation when started irrigating with wastewater.

3.4 Methods

The general design had been accomplished for the construction of wetland by following the stipulated design features. After the growth of the reeds, it was charged with the wastewater and efficiency was measured in terms of selective parameters.

3.5.1 Need Assessment Survey:

Before starting the treatment of wastewater, an exhibition was organized and questionnaire survey was done with the visitors orally and by filling questionnaire forms in order to know their concern about biological wastewater treatment using phytoremediation technology.

3.5.2 Morphological analysis

The morphological changes in the plants were studied at every 15 days since the day of plantation. The morphological changes were recorded on average height and hedge development for Vetiver and in addition number of shoots grown in case of common reed as the pattern of their reproduction and development was different. The number of dead plants and yellowing or drying of leaves was also recorded.

3.5.3 Laboratory analysis

The standard operating procedures were followed to determine the physical and chemical parameters of wastewater samples collected at an interval of 15 days and each samples were preserved in refrigerator for 24 hours.

Physical parameters of wastewater

a) Temperature:

Measurement of Temperature was done by dipping Mercury filled celsius thermometer. Calibration with another thermometer of known accuracy was done to minimize the error (APHA-AWWA-WPCF, 2005).

b) pH:

Measurement of pH with electrometer is accomplished by determining the potential developed by an electrical cell. The cell consists of a glass electrode system immersed in a test solution. The electrode system is pH sensitive and develops an electrical potential linearly proportional to the pH of the solution in which it is immersed (4500 - H^+ B: APHA-AWWA-WPCF, 2005).

c) Conductivity:

Conductivity is measured by Solution of electrolytes conducts an electric current by the migration of ions under the influence of an electric field. In the test solution, electrical conductivity at the laboratory temperature shall be used and indicated by michromhos per cm (1µmhos / cm = 0.01µS / cm). The measured conductance corresponds to a reciprocal number of the resistance of the solution (2510 B, APHA-AWWA-WPCF, 2005).

Chemical parameters of wastewater

a) BOD:

Determination of BOD by Winkler iodometric Modification (Dilution & Seeding) is done by neutralization of the sample of water to be analyzed and dilution with varying amounts of dilution water rich in dissolved oxygen containing a seed as desired. A completely filled and stoppered bottle is incubated at a controlled temperature for five days at 20^oC in the dark. DO concentration before and after incubation is determined. Finally, the BOD is computed from the difference between initial and final DO (5210 B, APHA-AWWA-WPCF, 2005).

b) COD:

Reflux in the presence of mercuric sulfate of a test portion with a known amount of 1N potassium dichromate and silver catalyst in strong sulfuric acid for two hours, during which the oxidizable materials present reduce part of the dichromate. The remainder of the dichromate is titrated of with ammonium ferrous sulfate. The amount of dichromate consumed shall be obtained to express the COD_{Cr} by the corresponding amount of oxygen mg/l (5220 B, APHA-AWWA-WPCF, 2005).

c) Nitrate:

The absorbance of the filtered sample is taken at 275 and 220nm respectively. To obtain the absorbance due to nitrate ion, subtract two times the absorbance reading at 275nm from the reading at 220nm of samples and standards. Finally, the nitrate concentration of the sample is calculated from the calibration curve (4500 - NO_3^- B, APHA-AWWA-WPCF, 2005).

d) Total Phosphorous:

Ammonium molybdate and potassium antimonyltartarate react with the orthophosphate formed after sulfuric acid – nitric acid digestion in acidic solution to form antimony phosphomolybdate complex (heteropolyacid-phosphomolybdic acid) which is reduced to intensely blue complexes by ascorbic acid. Finally the absorbance of color compound is measured in spectrophotometer at 880nm (APHA-AWWA-WPCF, 2005).

e) Chloride:

The presence of chloride ion in water and wastewater can be detected using the argentometric method, in which standard silver nitrate is used to titrate the sample. The chloride ion is quantitatively precipitated as white silver chloride. Since the silver chloride in nearly invisible, the indicator potassium chromate is used to observe the end – point (3500 - Mg B, APHA-AWWA-WPCF, 2005).

f) Free Carbon Dioxide:

Free carbon dioxide reacts with sodium hydroxide to form sodium bicarbonate. The completion of the reaction in the tittration is indicated by the development of the pink color characteristics of phenolphthalein indicator at the equivalence pH of 8.3 (4500 - CO_2 C: APHA-AWWA-WPCF, 2005).

Microbial parameter of wastewater

a) **Total Coliform Count:**

The total number of bacteria formed on culture after passing through membrane filter was counted and the results were expressed as MF Index / 100ml (9221 B, APHA-AWWA-WPCF, 2005).

Analytical Parameters of soil samples

Soil analysis was done in the laboratory after air drying the soil sample at room temperature and grinding into the powder form.

a) **Organic Matter / Organic Carbon:**

Organic matter was determined by Modified Walkley & Black titration method. The organic matter / carbon in the soil sample are determined by wet oxidation method. The soil sample treated with potassium dichromate in acidic condition is heated to 150^oC for 30 minutes. The amount of organic carbon in the sample can be determined by measuring the amount of unreacted dichromate by titrating with standard ferrous ammonium sulfate (Manual on soil sampling & methods of analysis, 1978).

b) **Total Nitrogen:**

Total N includes all forms of inorganic N, such as NH_4, NO_3 and NH_2 (urea), and the organic N compounds such as proteins, amino acids and other derivatives. While organic N materials can be converted into simple inorganic ammoniacal salt by digestion with sulphuric acid, for reducing nitrates into ammoniacal form, the modified Kjeldahl method is adopted with the use of sodium thiosulfate. At the end of digestion, all organic and inorganic salts are converted into ammonium form, which is distilled and estimated by using standard acid (FAO Fertilizer & Plant Nutrition Bulletin No. 19, 2008).

c) **Available Phosphorus:**

Two procedures namely Oslen and Bary were assigned to determine the available phosphorus in the soil samples.

Oslen method:

This method estimates the relative bioavailability of inorganic ortho-phosphate (PO_4-P) in soils with neutral to alkaline pH. The method is based on the extraction of phosphate from the soil by 0.5 M sodium bicarbonate solution adjusted to pH 8.5. In the process of extraction, hydroxide and bicarbonate competitively desorbs phosphate from soil particles and secondary absorption is minimized because of high pH. The orthophosphate ion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with abscorbic acid to form a blue complex, which absorbs light at 880 nm (Soil Analysis, Jackson *et al.,* 1982)

Bary method:

This method estimates the relative bioavailability of inorganic ortho-phosphate (PO_4-P) in soils with acid to neutral pH, using a dilute acid solution of hydrochloric acid containing ammonium fluoride. The orthophosphate ion reacts with ammonium molybdate and antimony potassium tartarate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 660 nm (Soil Analysis, Jackson *et al.,* 1989)

3.5 Data analysis

The obtained data were tabulated were tabulated in Microsoft Excel 2007 and various analysis technique like SPSS 20, R Programming 2.12.1 were taken into consideration which would be used for the main skeleton the of the report. Similarly, the standard operating procedures were considered for sampling and analysis of influent and effluent.

CHATER IV: RESULT

The water demand on the basis of information provided by store and account section of the college entailed that the daily wastewater production was about 2500 Litre. The daily-generated effluent (Organic + Chemical) of the college itself and nearby households and agricultural runoffs were not even primarily treated before discharged into nearby Khasyang Khusung Khola and possessed threats to the general river water quality and aquatic life followed by aesthetic values reduction. Besides irrigation, the people have been using the river water for their rituals values also.

4.1 Outcome of Need Assessment Survey

Altogether 250 visitors visited the site among which 90 were registered and 55 of them dropped their views about the wastewater treatment. They were asked orally and also some questionnaire forms were filled based on the information given by them. Among the visitors, 22 people already had knowledge about wastewater treatment and for the remaining it was new concept. 53 people said wastewater treatment is necessary to be done and 2 of them were not sure about it. All 55 people who dropped their views liked the model of wastewater treatment by phytoremediation using *Chrysopogon zizanioides* and *phragmites karka.*

4.2 Wetland maintenance and maturation

The raw wastewater was injected through a (2 inch diameter) plastic pipe PVC (buried) under the gravel lining with wire net in the first row of each pond in order to trap the solid wastes and allowing only water to reach to the plants roots by subsurface flow and the treated water passed out from the fifth row from the outlet pipe. The irrigation was done by fresh water for the first two month until the plants were grown well after that wastewater was used directly for irrigating plants.

4.3 Morphological changes in plants

The average growth of Vetiver for weeks 4, 8, 12, 16, 20 and 24 were 23±0.72cm, 45.6±9.24cm, 81.8±14.65cm, 140.6±13.97cm, 174.2±23.17cm and 231.4±10.32cm respectively. The average initial and net height changes were 6±0cm and 225.8±9.66cm. Likewise for Common reed ponds, these values were 62±9.55cm, 103.2±5.05cm, 131.4±4.82cm, 147.6±7.05cm, 171.8±8.18cm and 197.8±6.63 respectively. The average initial and net height changes were 32±0 and 164.6±7.35cm. Similarly they were 40±18.41cm, 42±20.38cm, 66±30.77cm, 94±24.08cm, 115±21.22cm and 140±23.94cm respectively in mixed pond. (Appendix IV)

Decay of old plants and yellowing of leaves was observed in Phragmites plants. On average 4-6 new plants emerged in each row of Phragmites and altogether 7 plants were completely decayed and the remaining were also showing the increasing number of yellowing of leaves during each observation. Vetiver showed the uniform growth, some leaves were dried but all the plants survived very well. The hedge growth was found increasing from 2cm during plantation to upto 19cm after six months. The hedge growth in the middle of later rows were very thin (5-10cm) compared to that of Vetiver pond alone. The average initial and net height changes were 36±15.61cm and 149.83±34.36 (Figure 4.1).

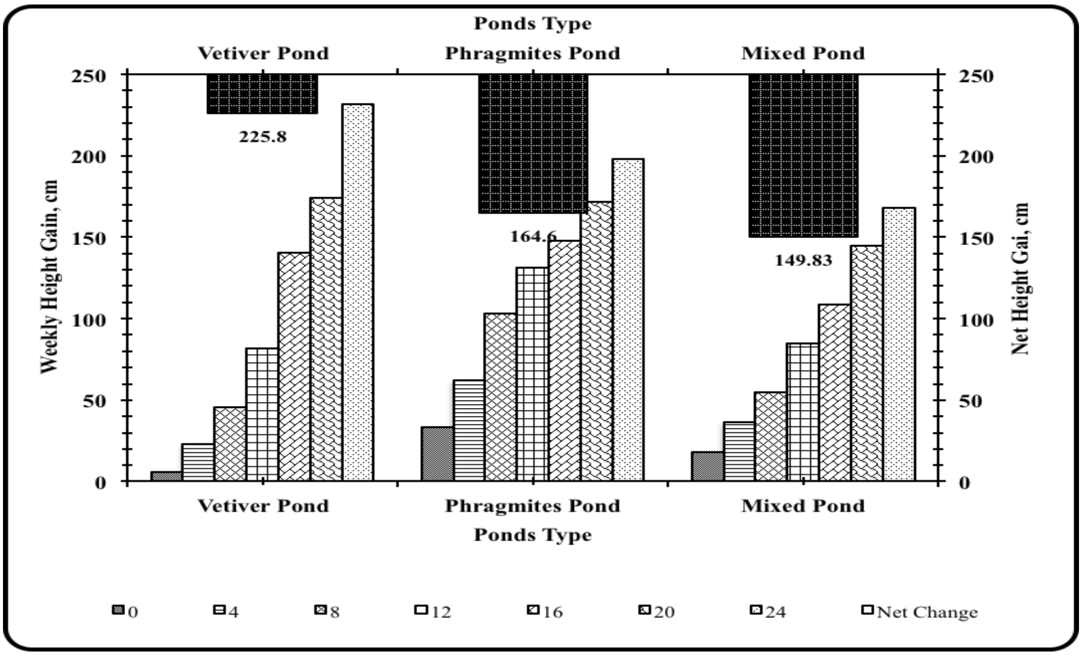

Figure 4.1: Reed Weekly Height Change inVarious Treatment Ponds

4.4 Physical parameters of wastewater after treatment

The physical parameters of wastewater showed slight change in the parameters like pH and Temperature as they were collected from nearby sample ponds with similar soil type and atmospheric condition. Electronic conductivity was decreased to normal level after treatment as compared between raw inlet wastewater and the treated water.

4.4.1 Temperature

The temperature change was independent of the type of treatment at the same sampling time rather it was found to be different in different weeks during the study period (July to September, Figure 4.2). The average temperature ranged from 19^{o}C (June) and was to about 26^{o}C (August).

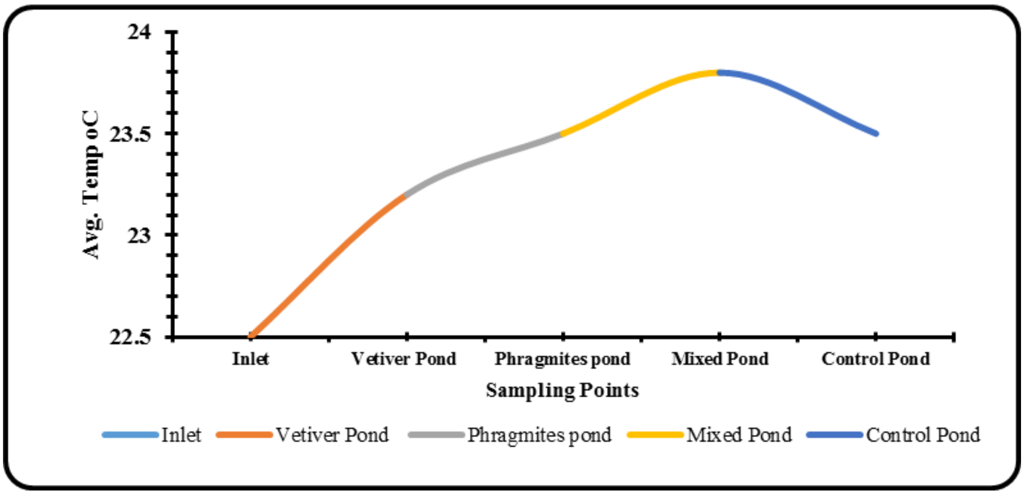

Figure 4.2 Average temperature change in different sample ponds

4.4.2 pH

The average pH range for the inlet raw wastewater was 6.4 to 7.7 (Table 4.2, Figure 4.3). Phragmites pond had the highest pH value 7.9 during second sampling on 21st June 2014. The variation of pH among the treatment ponds has been shown in the figure 4.3.

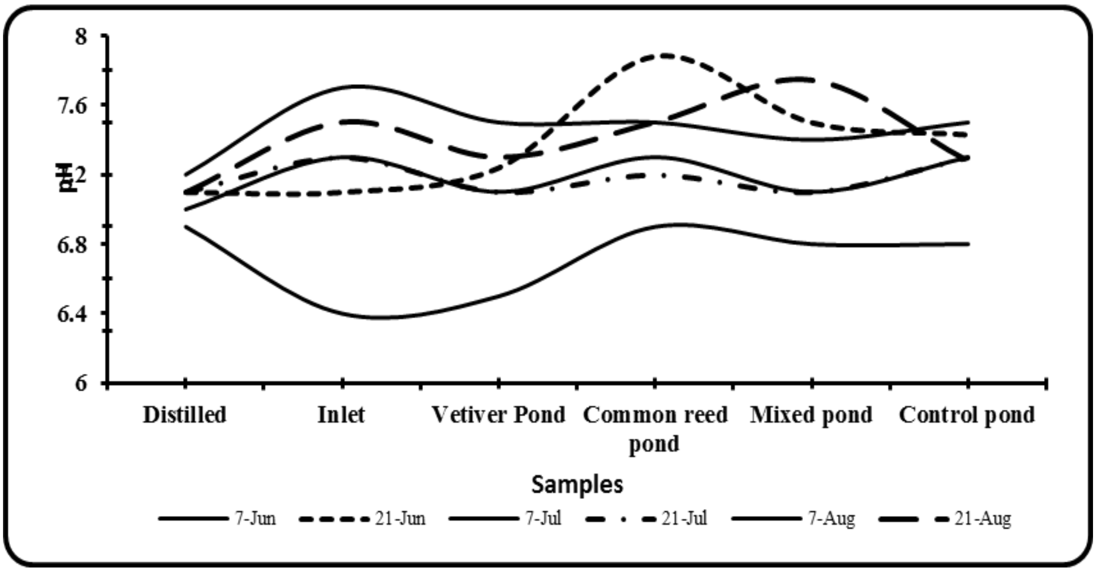

Figure 4.3 Variation of pH among the samples

4.3.3 Electronic Conductivity

The conductivity was recorded highest at the last sampling date (August, 2014:1090 µS/cm) at the inlet that decreased to 403 µS/cm by Vetiver pond, 521 µS/cm by Phragmites pond, 506 µS/cm by mixed pond and 802 µS/cm by control pond after treatment. The value of electronic conductivity of the inlet and different treatment ponds at different sampling time have been presented in the figure 4.4 below.

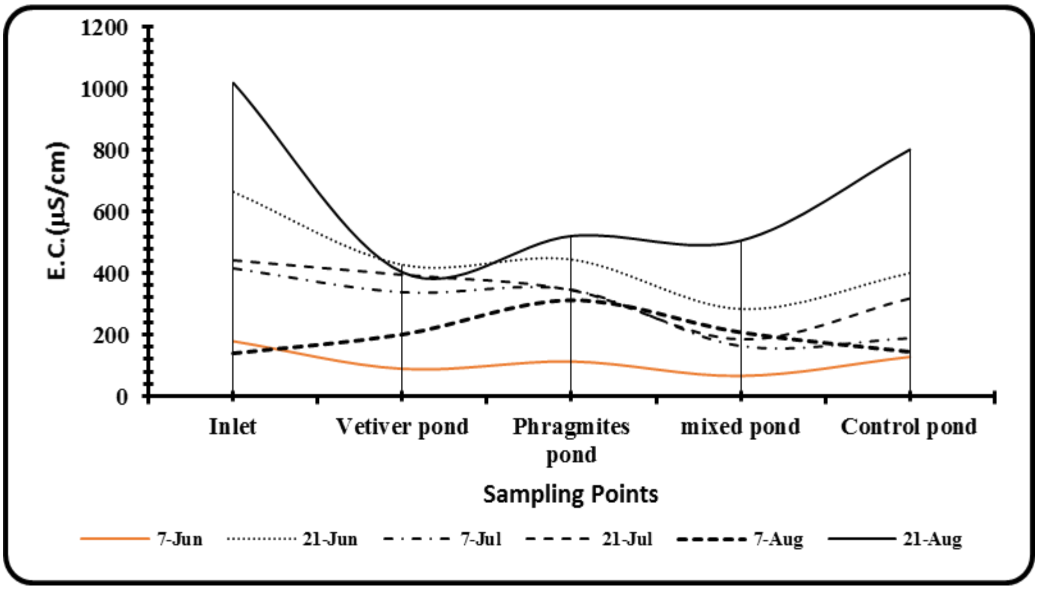

Figure 4.4 Change in Electronic Conductivity

4.5 Chemical parameters

The chemical parameters of wastewater before and after treatment were studied under following sub-headings.

4.5.1 Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

The value of BOD in the inlet sample during first sampling after three month of plantation in treatment ponds on 21st June, 2014 was found to be 104.69 mg/L which was reduced to 24.2 mg/L (76.88%) by Vetiver pond, 64.4 mg/L (38.49%) by Phragmites pond, 56.4 mg/L (46.13%) by mixed pond and 72.48 mg/L (30.76%) by control pond respectively. During the last sampling also the BOD concentration was found to decrease from 52.34 mg/L in the inlet to 8.0 mg/L (84.70%), 20.13 mg/L

(61.53%), 12.08 mg/L (76.92%) and 32.21 mg/L (38.46%) in the Vetiver, Phragmites, mixed and control ponds respectively.

The value of COD was 853 mg/L in the raw wastewater during the first sampling on 7th June, 2014 which was reduced to 267mg/L (69.51%) by Vetiver treatment pond, 608 mg/L (30.48%) by Phragmites treatment pond, 512 mg/L (41.46%) by mixed pond and 603 mg/L (31.09%) by control pond. The Maximum COD concentration was during the second and forth sampling on 21st June and 21st July, 2014. Its value was found to decrease from 960 mg/L in the inlet to 192 mg/L (80%), 533 mg/L (44.47%), 379 mg/L (60.52%) and 597 mg/L (37.81%) in the Vetiver, Phragmites, mixed and control ponds respectively. BOD and COD removal by the different treatment ponds have been mentioned in the figure 4.5 given below.

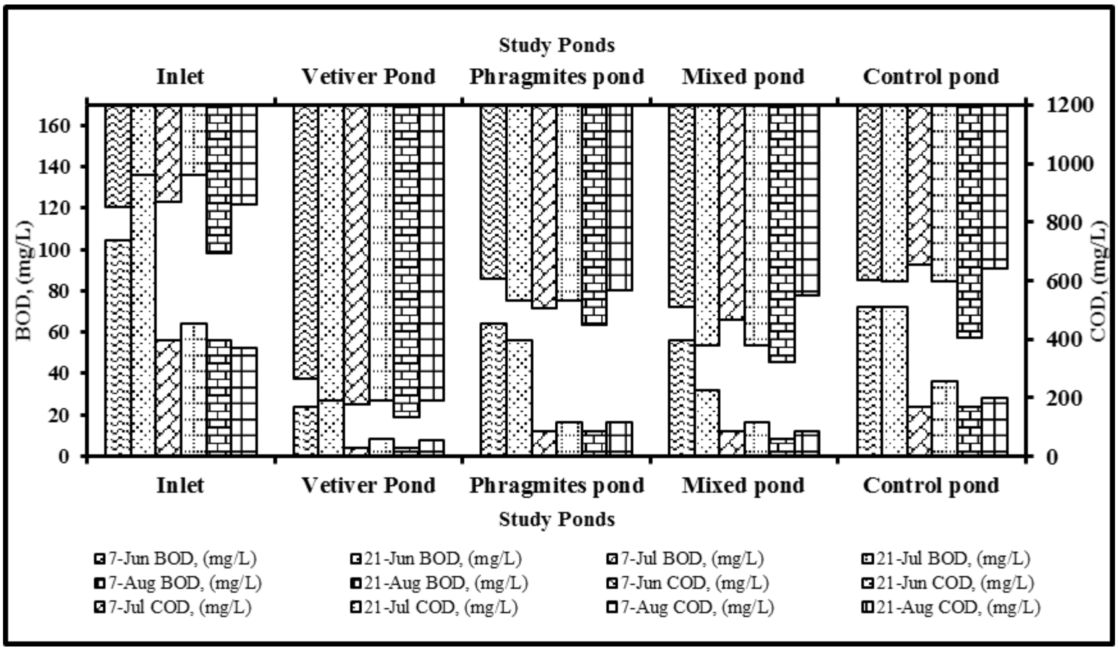

Figure 4.5 BOD and COD removal

4.4.3 Nitrate (NO_3)

The concentration of nitrate for the last sampling on 21st August, 2014 was obtained as 10.21 mg/L at the Inlet which was then reduced to 0.92 mg/L (90.98%) by Vetiver treatment pond, 1.85mg/L (81.88%) by Phragmites treatment pond, 1.62 (84.13%) mg/L by mixed pond and 7.15mg/L (29.97%) by control pond. The reduction in concentration has been shown more clearly in the figure 4.6 below.

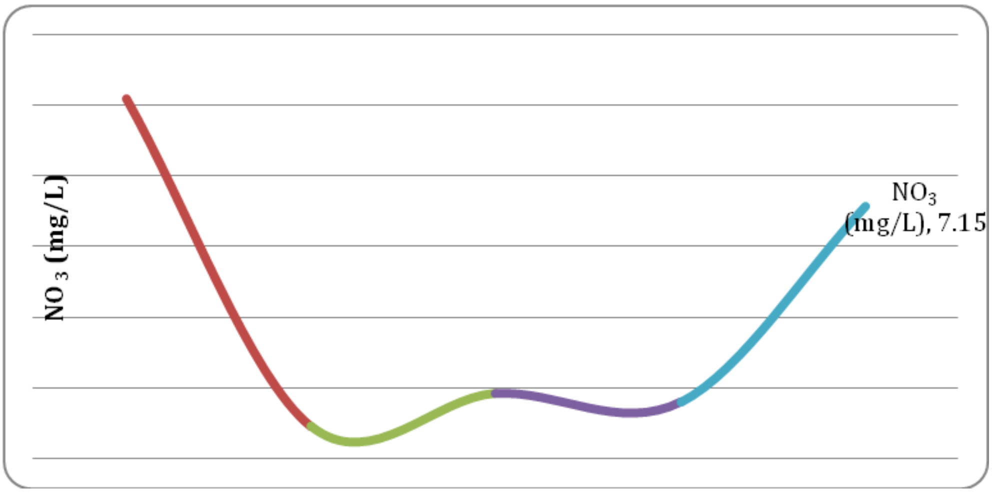

Figure 4.6 Nitrate concentration during the last sampling

4.4.4 Carbon Dioxide

The CO_2 Concentration was maximum 220 mg/L at the raw wastewater inlet during the last sampling on 21st August, 2014 which was then reduced to 140.8 mg/L (36%) by Vetiver treatment pond, 193.6 mg/L (12%) by Phragmites treatment pond, 176 mg/L (20%) by mixed pond and 211.2 mg/L (4%) by control pond respectively. The minimum value was obtained during third sampling on 7th july, 2014. The trend of reduction in the value has been mentioned in the figure 4.7 mentioned below.

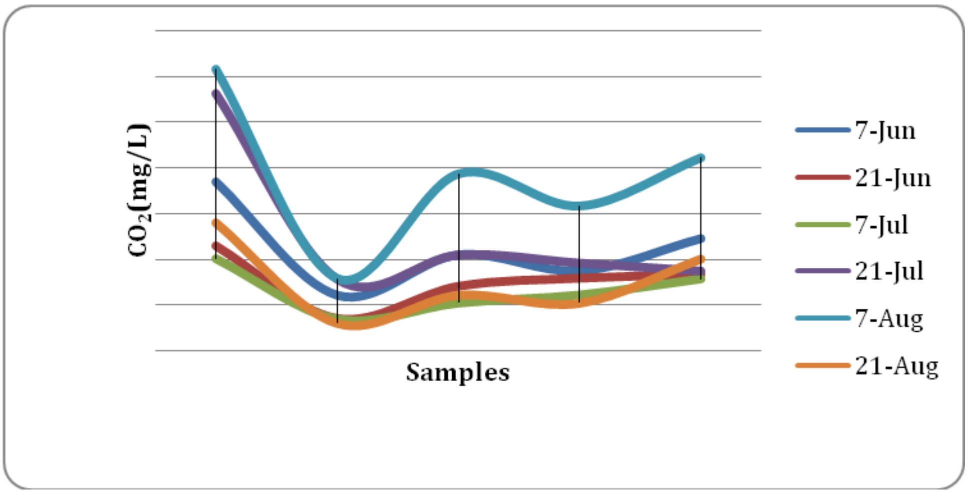

Figure 4.7 Carbon concentration reduction

4.4.5 Total Phosphorus and Chloride Content

The observed concentrations of total phosphorous in inlet, Vetiver, Phragmites, mixed and control ponds at the starting phase (7th June, 2014) were 29.41mg/ L, 10.72 mg/ L, 22.67mg/L, 17.77mg/L and 35.24 mg/L respectively. But the inlet concentration

(24.51 mg/L) at the end of study phase (21st August, 2014) was reduced to 3.06mg/L, 11.03 mg/L, 9.80 mg/L and 16.54 mg/L in the respective ponds.

During different study period from chloride removal after treatment of raw wastewater followed the trend of Vetiver > mixed > Phragmites > control ponds. The inlet concentration of chloride (233.2 mg/L) was reduced to 44 mg/L (Vetiver treatment pond), 110 mg/L (Phragmites treatment pond), 92.4 mg/L (mixed pond and) 171.6mg/L (control pond) respectively**.**

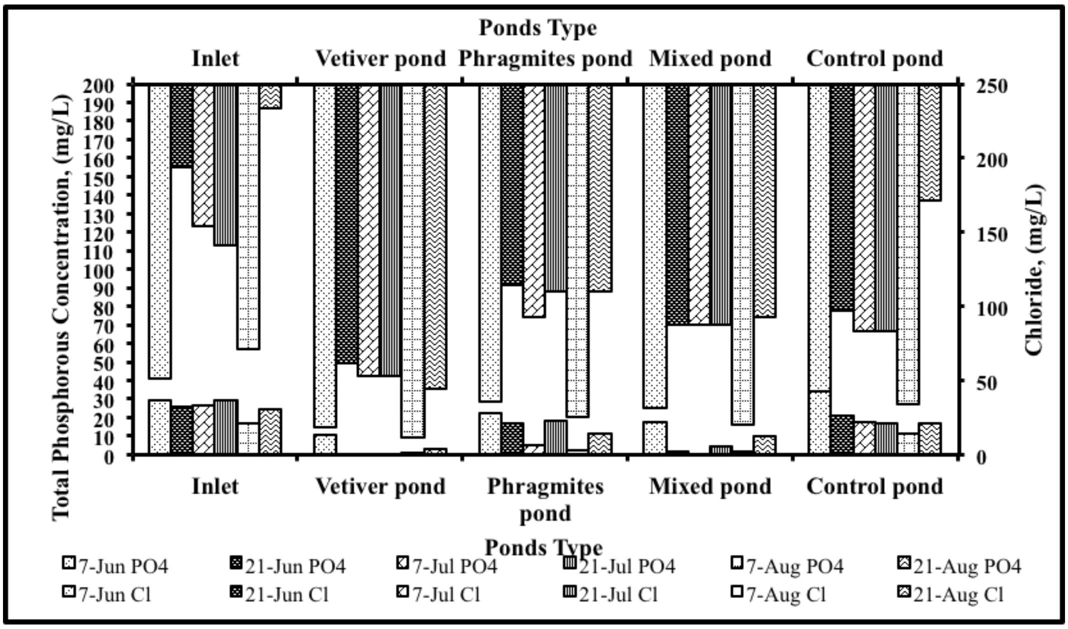

Figure 4.8 Total Phosphorus and Chloride reduction

4.5 Microbial parameter

4.5.1 Coliform Count

Coliform count was done by membrane filter suction and incubation of bacteria in the media for growth and it was found that the coliform count of the Inlet was >500 counts/100ml during mansoon i.e. July to August whose counts decreased to >300 counts/100ml which after treatment was reduced to 76-33 counts/100ml by Vetiver, 95-115 counts/100ml by Phragmites, 89-82 counts/100ml by mixed and >100 counts/100ml by the control ponds respectively on the sixth month of plantation.

4.6 Soil parameters

4.6.1 Organic Matter / Organic Carbon

The organic matter was determined by using Modified Walkley & Black method and measure of the amount of unreacted dichromate by titrating with standard ferrous ammonium sulfate gives the value of organic matter in the soil sample. The organic matter before the plantation in the prepared treatment ponds were 5.39% for Vetiver pond, 4.38% for Phragmites pond, 5.01% for mixed pond and 5.39% for the control pond which within three months after plantation showed significant change and the value decreased upto 3.36%, 2.98%, 3.61% and 1.90% for Vetiver, Phragmites, mixed and control ponds respectively after three months and 0.38%, 2.60%, 1.65% and 2.54% respectively after six months of plantation. The percent organic carbon also showed the similar decreasing trend in which Vetiver pond showed the highest decrease in the value of organic carbon from 3.13% to 1.95% after three months and 0.22% after six months followed by mixed pond, control pond and finally Phragmites pond. The decrease in the organic matter and organic carbon at different time interval has been mentioned in the figure 4.9 given below.

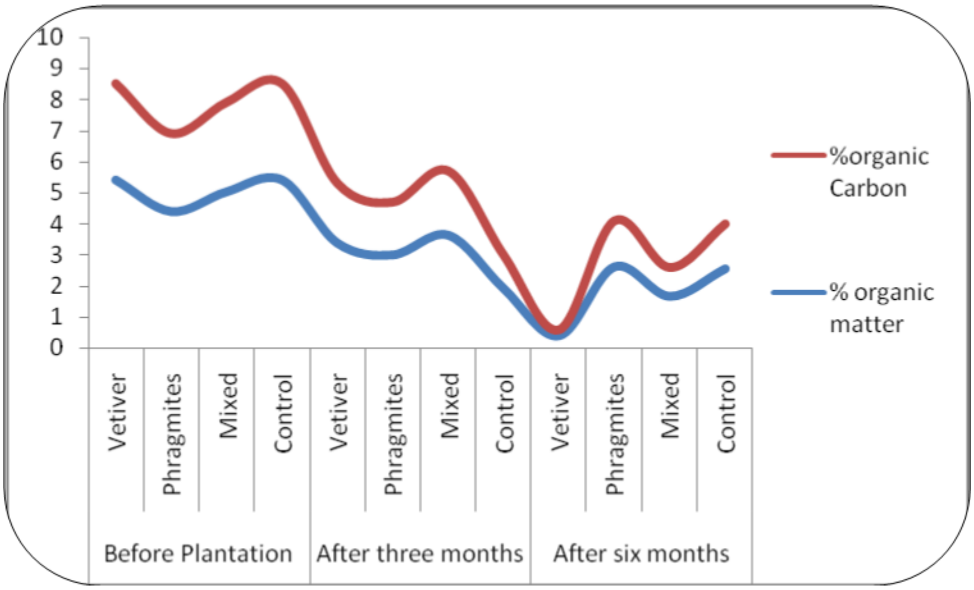

Figure 4.9 Changes in % Organic Matter and Organic Carbon

4.6.2 Total Nitrogen

The Nitrogen content in soil showed decreasing value as compared to that of before plantation. The vetiver pond sample showed the decrease from 0.24% before plantation to 0.04% after six months. Similarly, the value was in decreasing order for Phragmites, Mixed and Control ponds among which Mixed pond showed the better result after Vetiver. The % Total Nitrogen in the Mixed pond before plantation was 0.22%. The Value decreased to 0.17% after three months and finally reached 0.09% after six months of plantation in which both Vetiver and *Phragmites karka* were planted in alternate rows. The trend of change in the Total Nitrogen % has been mentioned in the figure 4.10 given below.

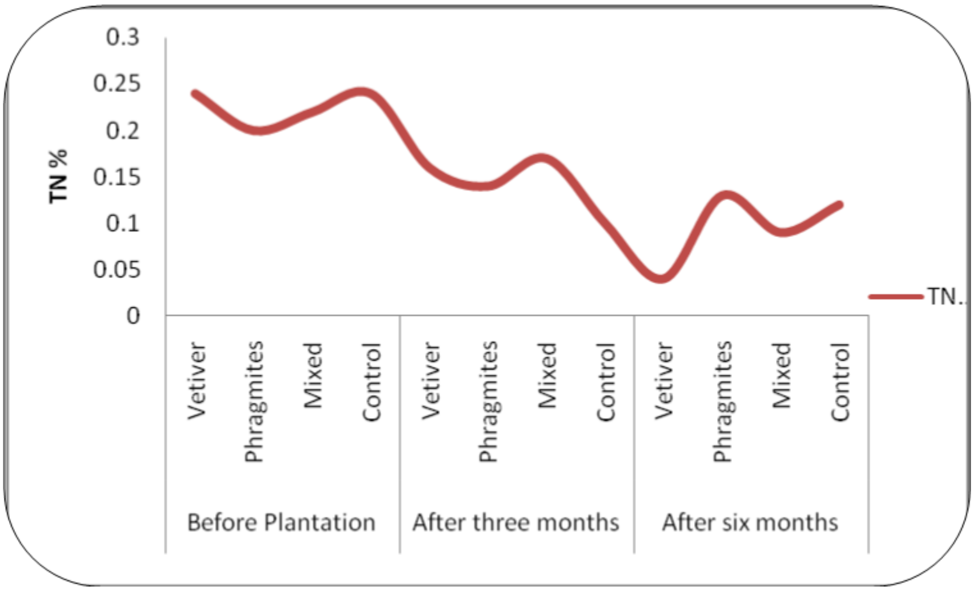

Figure 4.10 Total Nitrogen change with time

4.6.3 Available phosphorus

Total available Phosphorus was estimated using Oslen and Bary's method. This value gives the measure of available inorganic orthophosphate present in the soil which absorbs excess acid and helps to maintain soil pH. The initial value was high in Vetiver and Control pond i.e. 61.26ppm while that of mixed and phragmites ponds were 56.33ppm and 48.10ppm respectively. After six months of plantation in the treatment ponds, the values became -3.73ppm in Vetiver pond, 12.72ppm in mixed pond, 24.24ppm in control pond while in the Phragmites pond the value obtained was only 25.06ppm which was the greatest of all. The change in Available Phosphorus concentration in soil at different time has been mentioned in the figure 4.11 given below.

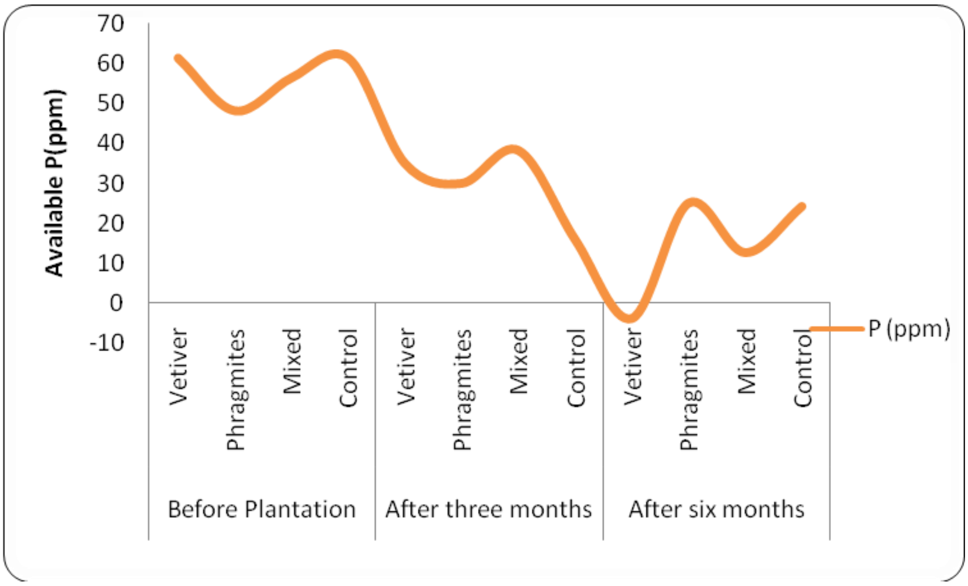

Figure 4.11 Available Phosphorus concentration with time

4.7 Relation between variables

4.7.1 COD removal efficiency

High significant difference was obtained between the wastewater treatment by Vetiver and *Phragmites karka* with T Value 22.7.6 and mean difference 39.87 while negative T value shown by the Paired t-test between *Phragmites karka* and Mixed treatment pond in reducing COD concentration which shows that mixed pond showed the better performance in reducing COD from the wastewater. Similarly, The positive T value 9.59 and mean difference 28.75 shows that Vetiver performed better tham mixed pond in reducing COD concentration from wastewater.

Table 4.1 Paired T- Test for COD reduction

Compared Between	T	DF	P-Value	95% confidence interval		Sample mean diff
V Vs P	22.706	5	3.08e-06	35.35626	44.38374	39.87
V Vs M	9.5919	5	0.0002087	21.04633	36.45700	28.75167
P Vs M	-3.663	5	0.01455	-18.920748	-3.315918	-11.11833

4.7.2 COD reduction efficiency of Vetiver with growth

The increase in growth rate of Vetiver plant didn't show significant difference in the F-value. The minimum F- value 0.112 of Vetiver show that the COD removal efficiency was irrespective of its height growth. As the sample test was only done after three months, the plants had growth to their saturated height by that time. This shows that the treatment done by Vetiver was almost of the same range i.e. 77.91% to 81.70% reduction from the initial value.

Table 4.2: One way ANOVA of COD reduction effeciency of Vetiver with growth rate

Vetiver	Df	Sum Sq	Mean Sq	F value	Pr(>F)
(COD Red %)	1	16.1	16.06	0.112	0.754
Residual	4	571.9	142.99		

4.7.3 COD reduction efficiency of *Phragmites karka* with growth

The increase in growth rate of *Phragmites karka* didn't show significant difference in the F-value but still the growth of plant had slight influence in wastewater treatment efficiency by *Phragmites karka* as shown by the F value 1.614. The Growth pattern of *Phragmites karka* was not uniform as that of Vetiver. There were continuous drying of old plants and emergence of the new ones which has impact on the wastewater treatment efficiency of the treatment plant. The COD reduction efficiency of *Phragmites karka* was found in the range i.e. 34.48 % to 48.88 % reduction from the initial value.

Table 4.3: One way ANOVA of COD reduction effeciency of *Phragmites karka* with growth rate

Phragmites karka	Df	Sum Sq	Mean Sq	F value	Pr(>F)
(COD Red%)	1	636.4	636.4	1.614	0.273
Residual	4	1577.0	394.2		

4.7.4 ANOVA of soil organic matter VS percent change in organic carbon

The result from the ANOVA test shows that with the decrease in organic matter in the soil, the percentage organic matter also decreases. These organic matter are the food for the plants for their growth which is available as nutrient in the form of various organic compounds which is produced either by death or decay of living organisms or by atmospheric carbon absorption by plants and soil microorganisms.

Table4.4 ANOVA of Organic matter VS percent change in organic carbon

	DF	Sum Sq	Mean Sq	F	Pr(>F)
Org. Matter	1	9.20	9.20	1.67e+20	<2e-16 ***
Org. Carbon	1	27.36	27.36	1.67e+20	<2e-16 ***
Residuals	10	0.00	0.00		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '

4.7.5 Reduction in COD concentration and soil organic carbon

With the increasing growth of plant COD was absorbed and so was soil organic carbon which is clearly observed from their reduced value. When COD was reduced by 69.51% by Vetiver, soil organic carbon was 1.95% and when the treatment efficiency was decreased i.e. reducing 77.64% of COD, the soil organic carbon was 0.22%. Similar when COD reduction by Phragmites was 28.72%, its soil organic Carbon was 1.73% and when it was 34.22% on the last month soil organic carbon concentration was decreased to 1.51%. The COD reduction by mixed pond 36.08% in the sixth month and soil organic carbon 0.95%. Control pond also showed similar trend which been described in the figure 4.12 mentioned below.

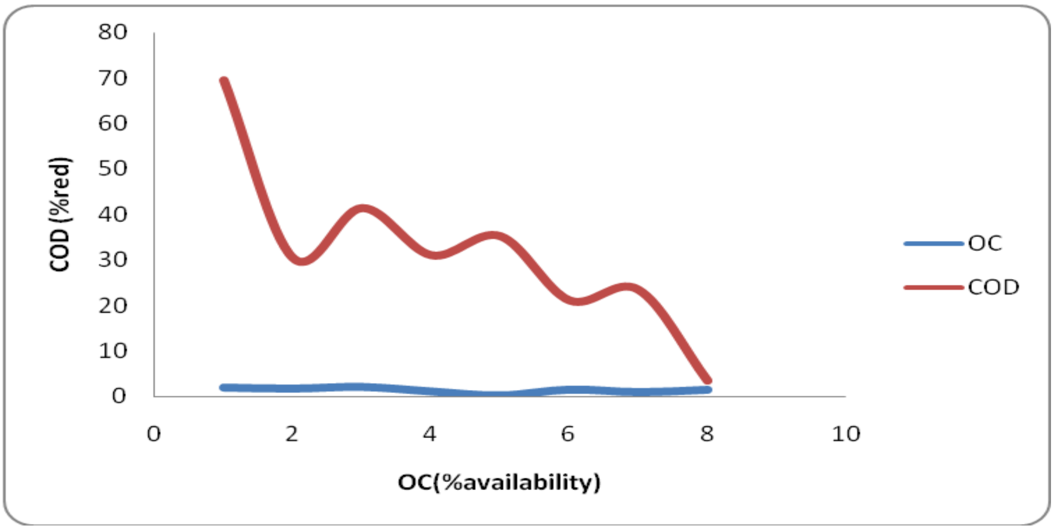

Figure 4.12 COD reduction% and Soil Organic Carbon %

CHAPTER V: DISCUSSION

5.1 Morphological Features

The average initial and net height changes of Vetiver were 6±0cm and 225.8±9.66cm. Likewise for Phragmites ponds, these values were 32±0 and 164.6±7.35cm. The average initial and net height changes were 36±15.61cm and 149.83±34.36 for mixed pond. On average 4-6 new plants emerged in each row of Phragmites. Altogether 7 plants were completely decayed and the remaining were also showing the increasing number of yellowing of leaves during each observation. Vetiver showed the uniform growth and all plants survived very well.

Also interesting fact is that the weed plants like *Alternanthera conyzoides* were found to grow more in Phragmites planted pond than the Vetiver planted one. Earthworms were found growing and reproducing thus making the soil more soft and porous, the quality of construction waste refill land has changed into fertile shiny clayey soil in which the agricultural activities done may give better production.

In an experiment for economic incentive of using Vetiver for essential oil extraction, the survival rate, plant height and biomass of Vetiver grass under different treatments of Pb, Zn and Cu treatments was 100 percent, except when 8000 mg Pb kg^{-1} of dry soil were used. (Danh, L.,Truong, P. and Foster, N., 2009). When growing under wetland conditions, Vetiver had the highest water use rate because of its extensive root penetration and fast growth compared with other wetland plants such as *Iris pseudacorus, Typha spp.*, *Schoenoplectus validus*, *Phragmites australis*. It has been estimated that Vetiver has7.5 times more water than *Typha* (Cull *et al.*, 2000).

All the above examples suggest Vetiver has extensive root penetration and fast growth rate in wetland condition compared to other plants used for phytoremediation. In my research, under similar condition of temperature, nutrient and volume of wastewater, Vetiver showed proper growth than *Phragmites karka* (Common reed).

5.2 Physico-chemical parameters

Result obtained from the lab analysis of different parameters is discussed as below:

Temperature impacts both the chemical and biological characteristics of water. Increased temperature accelerates the chemical reaction in water which affects the dissolved oxygen level in water and also implies the taste and odor (APHA, 1995). The temperature of the samples collected from different sample points at the same time were found to be in the same range as the sample plots were all in the same study sites. The temperature change was independent of the degree of treatment at the same sampling time rather it was found to be different in different sampling time.

pH indicates the intensity of acidity or alkalinity condition of any solution. The water greater than 8.5 or less than 6.5 are regarded as polluted (Trivedy and Goel, 1986). They cause corrosion in pipes and release toxic materials. The pH of the water samples during the experiment were within the acceptable limit. The average pH range for the inlet raw wastewater was 6.4 to 7.7. Phragmites pond had the highest pH value 7.9 during second sampling. Higher value of pH indicates that the water was basic and the higher value represents that water was not treated properly by Phragmites pond which can also be verified by the appearance of yellowish colouring and dying of Phragmites plants/ The pH range of samples from Vetiver pond were within range 6.5 to 7.5 which ia an acceptable normal range.

The conductivity increases with the increase in degree of pollution as there is increase in prevalence of dissolved salt and solids. The water with conductivity more than 20µS/cm is not suitable for irrigation (Trivedy and Goel, 1986). The electronic conductivity in the Vetiver pond and mixed pond after treatment was found decreasing when compared to Phragmites pond and control pond. The percent reduction of electronic conductivity was 60.45% by Vetiver, 48.87% by Phragmites, 50.34% by mixed and 21.29% by control pond respectively in the final sampling on 21st August, 2014. Though the values were not within acceptable limit for agriculture but compared to inlet it was highly decreased after being treated by plants in the treatment ponds.

The dissolved oxygen level contained in water is necessary for the survival of aquatic creatures which consume the chemical and biological contaminating agents as a source of their food so help in maintaining the water quality. The Value of BOD was found to be 136.91mg/L in the inlet sample during sampling of 21st June, 2014 while it was reduced to 32.21 mg/L (76.47%) by Vetiver treatment pond, 56.37 mg/L

(58.82%) by Phragmites treatment pond, 32.21 mg/L (76.47%) by mixed treatment pond and 72.48 mg/L (47.05%) by the control pond. During the last sampling also the BOD concentration was found to decrease from 52.34 mg/L in the inlet to 8.02 mg/L (80.76%), 16.21 mg/L (35.38%), 12.08 mg/L (53.84%) and 28.18 mg/L (41.53%) in the Vetiver, Phragmites, mixed and control ponds respectively. The treated values comply within the Nepal's national guideline value for irrigation i.e. <15mg/l while by the control pond it was reduced to 24.16mg/l. (NWQS, 2008)

Study of prototype reed bed using *Vetiver*ia *nigritana* (the Nigerian species) and *Phragmites karka* (Common reed) and the result showed reduction of BOD 82.0% and 85.0% (Badejo *et. al*., 2011). In a study on comparative studies of Vetiver grown in domestic wastewater from the Royal Irrigation Department community revealed that 'Monto' cultivar type of plot exhibited the highest ability to reduce: BOD 75.28%. The efficiency of wastewater treatment was found to increase with the age of Vetiver plant, and the highest was at 3 months of age (Chomchalow, 2006). Vetiver grass performed better than *Phragmites mauritianus* in removing of pollutants from domestic type of wastewater from the University main Campus of Dar es Salaam and found that: the organic removal (BOD) was on average 67.47% and 61.85% for Vetiver and *Phragmites mauritianus* grass respectively (Njau and Mlay, 2003). In an experiment in Toogoolawah sewerage plant in Australia, The BOD concentration was reduced from 120 mg/L to 29mg/L which was close to licence limit as prescribed by Environment protection Agency (EPA) 20mg/L (Ash and Troung, 2001).

Like above researches, during my research also, the rate of wastewater treatment was found to increase from the first sampling to the last in case of Vetiver pond but in the other hand it was in decreasing order for Phragmites pond and mixed pond. It may have occurred due to the dying and regrowth of Phragmites plant during their development which affected their efficiency of nutrient absorption. In control pond pond also the BOD treatment rate decreased slightly as the local grasses in control ponds were also dying and flourishing again. Thus, Vetiver was found to reduce BOD concentration to minimum limit and in some case close to licence limit as prescribed by the respective government or any agencies. As of my research, the BOD concentration after treatment by Vetiver was 8.02 mg/L which is within Nepal's national guideline value for irrigation i.e. <15mg/l.

COD is the measure of chemical concentration that is present in the water samples and gives the measure of its toxicity whether being suitable or not for drinking, irrigation and other various purposes. <400mg/L is the limit for protection of aquatic lives as proposed by Nepal Water Qaulity Guidelines. Above this level, the water is unsuitable for the survival of aquatic lives or conducting organic farming (NWQS, 2008). The Maximum COD concentration was during the third sampling on 7th July, 2014. Its value was found to decrease from 960 mg/L in the inlet to 202.66 mg/L (78.88%), 490.66 mg/L (48.88%), 426.66 mg/L (55.55%) and 570.66 mg/L (40.55%) in the Vetiver, Phragmites, mixed and control ponds respectively.

Vetiver and common reed *(Phragmites maritianus)* when experimented in separate plots to treat domestic type of wastewater from the University main Campus of Dar es Salaam and found that Vetiver grass performed better than *Phragmites mauritianus* in removing of pollutants COD of 37.9% and 46.2% for Vetiver and *Phragmites mauritianus* grass respectively (Njau and Mlay, 2003).The results of another research conducted by Aratha *et.al.*revealed that the wastewater treatment plant using *Phragmites karka* in reveals that the removal rate of COD 94%during the period of 1997-2000 AD and it was 84% during (2002-2003)AD (Aratha *et al.,*2003).

The results from my research were contrary to the above researches which show Phragmites showed more COD reduction potential than Vetiver. The reduction of COD remained almost the same for Phragmites but the soil organism was in increasing trend.

Nitrate represents the oxidized form of nitrogen. The most important source of nitrate are the biological oxidation of nitrogenous substances. The natural level of nitrate (NO_3-N) in water is 0.1mg/L. No_3-N has its standard guideline value between 0.2-10mg/l for pretection of aquatic life (NWQS, 2008). The concentration of nitrate for the last sampling on 21st August, 2014 was obtained as 10.21 mg/L at the Inlet which was then reduced to 0.92 mg/L. During this experimental research, the concentration of nitrate in the raw wastewater was 10.21mg/L which is very high in comparison to its natural limit in water resources. Trivedy and Goel (1986) say that Domestic and agricultural runoff wastewater contain highest concentration of nitrate. This is due to the use of ammonia and nitrogenous fertilizers while agricultural food production or their natural presence in soil as N- compounds are the essential nutrients for the agricultural production. While under treatment, the concentration of nitrate was reduced from 10.21 mg/L in the inlet to 0.92mg/L by Vetiver treatment pond, 1.85mg/L by Phragmites treatment pond, 1.62 mg/L by mixed pond and 7.15mg/L by control pond.

In an application of removal of N and P at a nutrient rich site, Vetiver could remove up to740 kg N $ha^{-1}$and 110 kg P ha^{-1}over 3 months at a nutrient-rich site (Vieritz *et al.*, 2003) which was much more than Rhodes grass, Kikuyu grass, Green Panic, Forage Sorghum, Rye grass, and Eucalyptus trees (Truong, 2003).

In my research also the nitrate concentration was reduced to very lower limit which indicated nitrogen fixation or the conversion of organic nitrate to different forms of nitrogen cycle which also plays vital role in soil fertility and productivity.

Phosphorus occur in water in the form of phosphate or the inorganic phosphorus which are collectively termed as total phosphorus. The higher concentration of phosphorus in water is indicative of pollution when it is above the range 0.005- 0.20 mg/L (ENPHO, 1997). The Total Phosphorus was obtained 24.51 mg/L at the inlet point during the last sampling on 21st August, 2014 which was reduced to 3.06mg/L (87.5%), 11.03 mg/L (55%), 9.80 mg/L (60%) and 16.54 mg/L (32.5%) in the Vetiver, Phragmites, Mixed and Control ponds respectively.

Australian research on Beelarong community farm showed that after five months of growth, the wastewater after passing through 5 rows of Vetiver had its total N reduced by 99% (from 9.3- 0.7 mg/l), total P by 85% (from 1.3-0.2mg/l) and fecal coliforms by 95% (from 500 to 23 organisms/100ml) (Troung and Hart, 2001).When Vetiver was used to treat the polluted river in central China, the removal percent of total P was 93.7% and 99% in two and four weeks and that of total N was 58% and 71% respectively (Annon, 1997; Zheng *et al.,* 1997).

Compared to the above experiments, the reduction percent of Phosphorus was also in the similar range (87.5%) after six month of growth. Slight difference might occur due to different atmospheric climate, soil type and type of raw wastewater fed. Compared to Vetiver, Phragmites showed very poor reduction of Phosphorus. This might be because of plant property or due to irregular growth and development of Phragmites.

Chloride in water originates from natural resources in the form of NaCl, KCl, $CaCl_2$ etc. Excessive chloride concentration increase the rate of corrosion in the water pipes. When present in useful amount, they help in purification of water against infectious germs and make it suitable for drinking, fishery, irrigation and other various purposes as chlorine is an essential nutrient required by all the aquatic creatures whether plants or animals and also for human beings as well. Chloride tolerance limit for aquatic life survival is <600mg/l and for aquaculture and Irrigation is 1 to 100 mg/l (Trivedy and Goel, 1986).

During different samplings of this research, the Chloride removal after treatment of raw wastewater followed the trend in which Vetiver treatment pond reduced maximum concentration followed by Mixed, Phragmites then control ponds. The Concentration values were obtained as 233.2 mg/L at the inlet whose values were reduced to 44 mg/L (81.13%) by Vetiver treatment pond, 110 mg/L (52.83%) by Phragmites treatment pond, 92.4 mg/L (60.37%) by Mixed pond and 171.6mg/L (26.41%) by Control pond respectively.

In pristine freshwater, chloride concentrations are usually lower than 10 mg/L and sometimes below 2 mg/L. The high concentration of chloride in this experiment might be due to the mixing of waste effluents from college toilets and Chemistry laboratory where various chemical had been used for conducting the practicals. The probable sources of chloride are weathering of some sedimentary rocks, sewage effluents, and agricultural and road run-off in an open drainage system.

Carbon Dioxide is the available carbon in the dissolved form in water which is utilized by plants in the form of their nutrients for growth and goes to the soil in the form of carbon compounds or the organic matter or to the atmosphere again by the means of atmospheric gas cycle. The CO_2 Concentration was maximum 220 mg/L at the raw wastewater inlet during the last sampling on 21^{st} August, 2014 which was then reduced to 140.8 mg/L (36%) by Vetiver treatment pond, 193.6 mg/L (12%) by Phragmites treatment pond, 176 mg/L (20%) by mixed pond and 211.2 mg/L (4%) by control pond respectively. The minimum value was obtained during third sampling on 7^{th} july, 2014.

The reduction percent of CO_2 concentration also determines the increase in soil organic matter and soil carbon. In my research the CO_2 reduction potential was highest for Vetiver followed by mixed, Phragmites then control ponds and increase of soil organic matter and organic carbon also showed the similar trend. This shows that the amount of CO_2 absorbed by plants from wastewater and atmosphere has been converted to other forms of carbon compounds which finally become component to take part in atmospheric Carbon cycle.

The efforts on wastewater treatment done in varoius areas in Nepal also show the satisfactory results. *Phragmites karka* was used as the primary absorbant during those reed bed treatment systems for the treatment of wastewater. Initial tests done in 1997 for performance evaluation of Reed bed system at Dhulikhel Hospital showed that the plant was able to remove 98% of total suspended solids (TSS), 98% of BOD5, 96% of COD, 99.9% of total coliforms, 80% of the ammonia nitrogen and 54% of phosphate. Aratha *et al.,* (2003) conducted follow up research for the same and the results revealed that the wastewater treatment plant using Phragmites karka in reveals that the removal rate of BOD, COD, TSS and PO_4 was 97%, 94%, 97% and 47% during the period of 1997-2000 AD and it was 96%, 84%, 93% and 41% during 2002-2003AD respectively. Another constructed wastewater treatment by reed bed system at Sunga, Thimi, Bhaktapur had an average BOD_5 of raw wastewater (1,775 mg/L). Monitoring of the performance of the system over its first year of operation shows that it removes organic pollutants highly efficiently up to 98% TSS, 97% BOD5 and 96% COD (Singh *et al*., 2007).

5.3 Microbial parameters

Microbial contaminants like coliform are the carriers of various kinds of water borne diseases like diarrhoea, dysentry, cholera and sometimes may result in severe effects like malnutrition, body disorders or even death. An exposure frequency of once every 10 years to flooding originating from combined sewers resulted in an annual risk of infection of 8%, which was equal to the risk of infection of flooding originating from rainfall generated surface runoff 2.3 times per year. (Man *et. al*., 2013)

Coliform count was done by Membrane filter suction and incubation of bacteria in the media for growth and it was found that the coliform count of the Inlet was >500 counts/100ml during Mansoon i.e. July to August whose counts decreased to >300 counts/100ml which after treatment was reduced to 76-33 counts/100ml by Vetiver, 95-115 counts/100ml by Narkot, 89-82 counts/100ml by Mixed and >100 counts/100ml by the control ponds respectively on the sixth month of plantation.

In my research the microbial concentration was found to reduce to very minimum value when compared to the raw inlet samples. The low microbial concentration shows that wastewater after phytoremediation contained less number of coliform and other bacterias which are carrier of various water borne diseases and thus were less prone to transmit diseases or cause infection.

5.4 Soil parameters

This increase in concentration of organic matter and percent organic carbon in soil supports that the carbon compounds that were present in dissolved form in water were consumed by the plants and soil organisms for their nutrition and thus they remain deposited in soil through uptake by plants for energy during their growth or go back to atmospheric cycle to make it available for other creatures as well.

The organic matter before the plantation in the prepared treatment ponds were 5.39% for Vetiver pond, 4.38% for Phragmites pond, 5.01% for mixed pond and 5.39% for the control pond within three months after plantation showed significant change and the value decreased upto 3.36%, 2.98%, 3.61% and 1.90% for Vetiver, Phragmites, mixed and control ponds respectively and 0.38%, 2.60%, 1.65% and 2.54% respectively after six months of plantation. This decreased value of organic matter may be due to uptake by plants during their growth. The percent organic carbon also showed the similar decreasing trend in which Vetiver pond showed the highest decrease in the value of organic carbon from 3.13% to 1.95% after three months and 0.22% after six months followed by mixed pond, control pond and finally Phragmites pond.

Nitrogen content in soil is the compound form of atmospheric nitrogen and the nitrogen compounds present with other chemicals in wastewater which are the useful nutrient source to other plants when they are available in favourable proportion in the soil.

The vetiver pond sample showed the reduction from 0.24% before plantation to 0.04% after six months. Similarly, the value was in decreasing order for Phragmites, Mixed and Control ponds.

The Available Phosphorus in the soil sample was found to increase with time after Vetiver and *Phragmites karka* were planted in the specific treatment ponds. The initial value was high in Vetiver and Control pond i.e. 61.26ppm while that of mixed and phragmites ponds were 56.33ppm and 48.10ppm respectively. After six months of plantation in the treatment ponds, the values became -3.73ppm in Vetiver pond, 12.72ppm in mixed pond, 24.24ppm in control pond while in the Phragmites pond the value obtained was only 25.06ppm which was the greatest of all. The reduction of total Phosphorus from wastewater was also in the similar range for the different treatment ponds.

Comparing the relationship among those parameters it was found that wastewater after passing through soil and plants, the toxic chemicals were absorbed and transformed to useful parameters by soil microorganisms and plant roots which were either used as food for plants and soil organisms or released back to the atmosphere by the mechanism of gaseous cycle.

5.5 Relation between variables

High significant difference between the wastewater treatment by Vetiver, *Phragmites karka* and mixed pondwith positive T value and mean difference 22.70 and 39.87 show that their is highly positive significance of effectiveness of Vetiver in COD concentration removal compared to the other two while negative T value (-3.66) shown by the Paired t-test between *Phragmites karka* and Mixed treatment pond in reducing COD concentration which shows that Mixed pond showed the better performance in reducing COD from the wastewater.

The increase in growth rate of Vetiver plant didn’t show significant difference in the F-value. As the sample test was only done after three months, the plants had growth to their saturated height by that time. This shows that the treatment done by Vetiver was almost of the same range i.e. 77.91% to 81.70% reduction from the initial value.

The increase in growth rate of *Phragmites karka* didn't show significant difference in the F-value but still the growth of plant had slight influence in wastewater treatment efficiency by *Phragmites karka* as shown by the F value 1.614. The Growth pattern of *Phragmites karka* was not uniform as that of Vetiver. There were continuous drying of old plants and emergence of the new ones which has impact on the wastewater treatment efficiency of the treatment plant. The COD reduction efficiency of *Phragmites karka* was found in the range i.e. 34.48 % to 48.88 % reduction from the initial value.

The result from the ANOVA test shows that with the increase in Organic matter in the soil, the percentage organic matter also increases. These organic matter are the food for the plants for their growth which is available as nutrient in the form of various organic compounds which is produced either by death or decay of living organisms or by atmospheric carbon absorption by plants and soil microorganisms.

The COD concentration in water and Organic Carbon percent in soil showed inverse relation when plotted in a graph i.e. with the decrease in the concentration of COD from water after treatment, the soil organic carbon concentration increases. When COD was reduced by 69.51% by Vetiver, soil organic carbon was 1.95% and when the treatment efficiency was decreased i.e. reducing 77.64% of COD, the soil organic carbon was 0.22%.

CHAPTER VI: CONCLUSION

Vetiver was found comparatively better than *Phragmites karka* in its morphology, growth and nutrient absorption. Though Phragmites plant is very tall, thick and have greater biomass than compared to Vetiver but even while planting together in the mixed pond, almost seven of the original *Phragmites karka* plant died and later new plant arised from it. After four month, the old tall *Phragmites karka* plants showed yellowing and drying of the leaves but it was not observed in that of vetiver, instead in the sixth month it showed its first enflorescence.

The pH and Coliform counts were within the permissible limit suggested by Nepal's National water quality Guideline and Canadian Ministry of Environment's Guidelines Values for irrigation, aquaculture and Protection of aquatic lives which is pH 6.5-9 and Coliform<1000/100ml.

Among the Physiochemical Parameters on the Sixth month of maximum growth, Vetiver, *Phragmites karka* and the mixed pond gradually reduced BOD(mg/L), COD(mg/L), Chloride(mg/L), $N0_3$ (mg/L) comply with the Guideline values set by Nepal water Quality Standards. Other parameters though their standard guideline value was not found in a very specific values during the literature review but their reduction performance was also shown maximum by the Vetiver, the mixed pond showed good performance than *Phragmites karka* followed by the control pond.

The obtained nutrient datas from the soil supports this result which showed reduction in organic matter, % organic carbon, Total Nitrogen and Average Phosphorus in the soil sample after third and sixth month of processing of wastewater treatment as the soil nutrients were utilized by plants for energy during their growth.

RECOMMENDATIONS

- More practical, reliable and cheaper method of treating effluent before being passed into the river should be sought; biological wastewater treatment would be one of the best alternatives.
- Wastewater treatment should be done at local level at every households, industries, hospitals and institution in small scale so that reuse and recycle can be done.
- Appropriate techniques for rainwater harvesting should be developed, particularly for major urban areas, as Urban runoff mixed with solid pollutants is the major cause of water pollution in the cities.
- Awareness activities about conserving water quality and quantity should be conducted in all parts of the country
- Strict laws and effluent standards should be enforced for the major contributors of wastewater like Industries, Hospitals, Hotels, Housings, Department malls etc.
- Guideline should be updated and maintained for the parameters and chemicals which have not been included in Nepal's National Water quality Guideline, 2008

REFERENCES

Adiningsih, J.S., Soejitno, J., Subowo, dan. (1998). Ameliorasi pencemaran agrokimia padalahan sawah intensifikasi Jalur Pantura, Jawa Barat. *Laporan Akhir RUT, Kantor NegaraRiset dan Teknologi, BPPN, DRN, LIPI,BPPT*. (In Bahasia Indonesia).

Adel E.EL-Leboudi (Soil Depat/. Faculty of Agriculture, Ain Shams University), Essam M. Abd-Elmoniem (Institute of Environmental Studies & Research, Ain Shams University), EzzatM.Solimanand Ola F. M. El-Sayed (National Organization for potable water & Sanitary drainage, Cairo), Removal of Some Heavy Metals from Treated WasteWater by Aquatic Plants, Egypt.

Anon. (1997). A consideration and preliminary test of using vetiver for waterultrophication control in Taihu Lake in China. Proc. Environmental group, Iinstitute of Soil Science, Academia Sinica, Nanjing. Proc. International Vetiver Workshop, Fuzhou, China October 1997.

APHA, AWWA, WEF. *Standard Methods for the Examination of Water and Wastewater*. (American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC, 2005).

Aratha, Tetsuji; "Wastewater in the Greater Kathmandu", Japan Association of Environment and Society for the 21st Century, March 2003.

Asghar, E., Ensiyeh, T., Mohammad, H.E., Sara, N., Fatemeh, J., Rahele, S., and Ali, F. (2013). Efficiency of Constructed Wetland Vegetated with Cyperus Alternifolius Applied for Municipal Wastewater Treatment. Journal of Environmental and Public Health, Volume 2013, Article ID 815962, http://dx.doi.org/ 10.1155/2013/815962

Ash R. and Truong, P. (2003). The use of vetiver grass wetland for sewerage treatment in Australia. Proc. Third International Vetiver Conference, Guangzhou, China, October 2003.

Asian Development Bank "Country Assistance Plan (2000-2002) Pipeline Update Nepal", June 2000.

Awuah, E., Oppong-Peprah, M., Lubberding, H.J. and Gijzen, H.J. (2004). Comparative performance studies of water lettuce, duckweed and algal-based stabilization ponds using low-strength sewage., J. Toxicol. Environ. Health-Part A., 67(20-22), 1727-1739.

Ayyasamy, P.M., Rajakumar, S., Sathishkumar, M., Swaminathan, K., Shanthi, K., Lakshmanaperumalsamy, P. and Lee, S. (2009). Nitrate removal from synthetic medium and groundwater with aquatic macrophytes., Desalination, 242, 286-296.

Bagmati Area Sewerage Construction/Rehabilitation Project (BASP). *The Implementation & Monitoring of the Bagmati Area Sewerage Construction/Rehabilitation Project (BASP).* Kathmandu: BASP, 2002 (brochure).

Badejo, A. A., Coker, A. O. and Sridhar, M. K. C. (2011). Tertiary Hospital Wastewater Treatment using Reed Bed Technology planted with V*etiveria nigritana* benth and P*hragmites karka* retz, Proceedings of the Environmental Management Conference, Federal University of Agriculture, Abeokuta, Nigeria, 2011, 497-504.

Billore, S.K., Singh, V.P., Nelson, R.M. and Dass, P. (2001). Treatment of mollases based distillery effluent in a constructed wetland in central india. Water science and technology 44 (11-12): 441-448

Bista, K.R., Khatiwada, N.R. (2004). Performance study on reed bed wastewater treatment units in Nepal, Department of Water Supply and Sewerage (DWSS) and School of Environmental Management and Sustainable Development (SchEMS).

Bramwell, S.A. and Devi Prasad, P.V. (1995). Performance of a small aquatic plant wastewater treatment system under Caribbean conditions., J. Environ. Manage., 44, 213-220.

Chaney, R.L., Malik, M., Baker, J.M., (1997). Phytoremediation of soil metals. ScienceDirect-Current opinion in Biotechnology- June 1997, Vol.8(3)

Chen, Z.M., Chen, B., Zhou, J.B., Li, Z., Zhou, Y., Xi, X.R., Lin, C. & Chen, G.Q. (2008). A vertical subsurface-flow constructed wetland in Beijing. Communications in Nonlinear Science and Numerical Simulation, 13(9) 1986–1997. doi:10. 1016/j.cnsns.2007.02.009

Chomchalow, N. (2006). Review and Update of the Vetiver System R&D in Thailand. Proc. Regional Vetiver Conference, Cantho,Vietnam.

Clayton, W.D., Harman, K.T. and Williamson, H. (2006). GrassBase - the online world grass flora. [Internet] Royal Botanic Gardens, Kew, United Kingdom.

Cooper, P.F., Job, G.F., Green, M.B., Shutes, R.B.E. Reed beds and constructed wetlands for wastewater treatment. Swindon, UK: Water Research Centre Publications, 1996. p. 154.

Cull, R.H., Hunter, H., Hunter, M. and Truong, P. (2000). Application of vetiver grass technology in off-site pollution control, (II) Tolerance of vetiver grass towards high levels of herbicides under wetland conditions., In: Proc. of the 2nd Intern, Vetiver Conference, Thailand.

Danh, L.T., Troung, P., Mammucari R., Tran, T., ad Fostar, N. (2009). Vetiver Grass, *Vetiveria Zizanioides*: A Choice Plant for Phytoremediation of Heavy Metals and Organic Wastes. *International Journal of Phytoremediation*, 11:664–691, 2009 Copyright C _ Taylor & Francis Group, LLC http://www.informaworld.com/smpp/title~content=t713610150

Darnal, Kiran; "Wastewater System in Kathmandu Valley: An Overview", *Development Insight*, August-October 2002.

Dong, C., Pang, Z., Xue, J., Liu, Y., Chen, J. and Zhang, R. (2012). Effect of screw excurtion pretreatment on pulps from chemical pulping. Peer reviewed article. Bioresources.com

Dierig, Sandra. (1999). *Urban environmental management in Addis Ababa: Problems, policies, perspectives, and the role of NGOs*. Hamburg: Institute of African Affairs. Esayas W. Meskel.

ENPHO, South Asian Trans-Boundary Water Quality Monitoring (SATWQM) Data, 2003.

ENPHO, Data from Dhulikhel Hospital Constructed Wetlands, 2002-2003.

EPA/600/R-99/107, Introduction to Phytoremediation, National Risk Management

Erakhrumen, A.A. (2007). Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Educational Research and Review, 2 (7):151-156.

Gamage, N.S. and Yapa, P.A.J. (2001). Use of water hyacinth[Eichhornia crassipes (Mart) solms] in treatment systems for textile mill effluents - A case study., J. Natn. Sci. Foundation Sri Lanka, 29(1&2), 15-28.

Ghimire, N. (2013). Change in Physiochemical parameters in different sites of Bagmati river. Case study.

Gupta, P., Roy, S. and Mahindrakar, A.B. (2012). Treatment of Water Using Water Hyacinth, Water Lettuce and Vetiver Grass - A Review. Resources and Environment 2012, 2(5): 202-215

Gurung, A. and Oh, S. (2012). An Overview of Water Pollution and Constructed Wetlands for Sustainable Wastewater Treatment in Kathmandu Valley: A Review Paper, Department of Biological Kangwon National University, South Korea, DOI: 10.5897/SRE12.053[Article Number: 8948E0D27900]. Vol.7(11), 1185-1194

Hammer, D.A. and Bastian, R.K. (1989). Wetland Ecosystems: Natural Water Purifiers. Chapter 2, pp 5 – 20 in D.A. Hammer (Ed.), *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural.* Chelsea, MI: Lewis Publishers.

Henry, J. R. (2000). In An Overview of Phytoremediation of Lead and Mercury. – NNEMS Report. Washington, D.C.; pp, 3-9.

Hillary G., Saik-Choon P. and Richards, A. (2003). Wastewater Treatment in Kathmandu, Nepal.

Irfan, A., Qureshi, Abdul, R. Ghumman and Hashim N. Hashmi. Performance Evaluation of Constructed Wetland for Wastewater Treatment. *Life Sci J* 2013. 10(4): 1139-1145]. (ISSN:1097-8135). http://www.lifesciencesite.com.

Kayranli, B., Scholz, M., Mustafa. A., Hedmark, A. (2010). Carbon storage fluxes within freshwater wetlands, A critical review. Wetlands 30, 111-124

Klomjek, P. and Nitisoravut, S. (2005). Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere. 58(5): 585-593. doi:10.1016/j.chemosphere.2004.08.073

Lambertini, C., Gustafsson, M.H.G., Frydenberg, J., Lissner, J., Speranza, M. and Brix, H., (2006). A phylogeographic study of the cosmopolitan genus Phragmites (Poaceae) based on AFLPs. Plant Systematics and Evolution 258(3–4): 161–182.

Mahommad, A.S., (2012). Municipal Wastewater Treatment Using Reed Bed Eco-Technology. *Resources and Environment 2012, 2(5)*: 202-215 DOI: 10.5923/j.re.20120205.04

Man, H.D., Berg, H.H.J.L.V.D., Leenen, E.J.T.M. , Schijven, J.F., Schets, F.M., J.C., Vliet, J.C.V.D, Knapen, F.V. and Husman, A.M.D.R. (2013). Quantitative assessment of infection risk from exposure to waterborne pathogens in urban floodwater, 1-10.

Mkandawire, M. and Dudel, E.G., (2007). Are lemna spp. effective phytoremediation agents?., Boremediation, Biodiversity Bioavailability, 1(1), 56-71.

Nepal Water Quality Standards, Government of Nepal 2008.

Njau, K.N. and Mlay, H. (2003). Wastewater Treatment and Other Initiatives with Vetiver Grass. 3rd International Vetiver Conference, Quangzhou China.

Oct 6-9, 2003. http://www.vetiver.org/ICV3-Proceedings/ TAN%20 wastewater.pdf.

Njau and Mlay (2003). Wastewater Treatment and other Research Initiatives with Vetiver Grass.

Shrestha, D. and Maharjan, S. (2009). Constructed Wetland: A Solution for Wastewater Treatment Hydro Nepal Vol. 5, pp 42-45

Shrestha, R.R., Tuladhar, B. and Shrestha, P. (2003). Experiences with Application of Constructed Wetlands for Wastewater Treatment and Reuse in Nepal, ENPHO Magazine, Environment and Public Health Organisation, Kathmandu, Nepal

Shrestha, R.R. (1999). Application of Constructed Wetlands for Wastewater Treatment in Nepal .A dissertation for the fulfillment of degree of the Doctor of Applied Natural Sciences. University of Agricultural Sciences, Vienna, Austria.

Shrestha, N., Lamsal, A., Regmi, B.L., Mishra, B.K. (2013). Current Status of water Environment In Kathmandu Valley, Nepal, Water and Urban Initiative Working Paper Series, Number 03, UNU - IAS

Singh, S., Haberl, R., Moog,O., Shrestha, R.R., Shrestha, P., and Shrestha, R. (2008) Performance of an Anaerobic Baffled Reactor and Hybrid Constructed Wetland Treating High-strength waste water in Nepal – A Model for DEWATS, Ecological Engineering 35 (2009); 654-660

Singh, S., Haberl, R., Shrestha, R.R., Shrestha, P. and Shrestha, R. (2007). Performance of the first community-managed constructed wetland in Nepal, Proceedings – International Conference on Sustainable Sanitation, Dongsheng, China

Singh, S. (2007). Constructed Wetlands Design, Construction, Operation and Maintenance Manual, *UN-HABITAT, 2008, Constructed Wetlands Manual, UN-HABITAT Water for Asian Cities Programme Nepal, Kathmandu.*

Singh, D., Tiwari, A. and Gupta, R. (2012). Phytoremediation of lead from wastewater using aquatic plants, Journal of Agricultural Technology 2012 Vol. 8(1): 1-11

Torodorovics. C., Garay. T.M., & Bratek, Z. (2005). The use of the reed (Phragmites australis) in wastewater treatment on constructed wetlands. Acta Biologica Szegediensis Volume 49(1-2):81-83, 2005

Trivedy, R.K. and Goel, P.K., (1986). Chemical and Biological methods of water pollution studies. Department of Environmental Pollution, Karad, India.

Truong, P. and Baker, D. (1998). Vetiver grass system for environmental protection., Technical Bulletin no. 1998/1, Pacific Rim Vetiver Network, Office of the Royal Development Projects Board, Bangkok, Thailand.

Truong, P.N. and Hart, B. (2001). Vetiver system for wastewater treatment. Technical Bulletin No. 2001/2. Pacific Rim Vetiver Network. Royal Development Projects Board, Bangkok, Thailand.

US EPA (1999). *Phytoremediation Resource Guide*, *EPA 542-B-99-003,* U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response Technology Innovation Office Washington, DC 20460

Vieritz, A., Truong, P., Gardner, T. and Smeal, C. (2003). Modelling Monto vetiver growth and nutrient uptake for effluent irrigation schemes. Proc. Third International Vetiver Conference, Guangzhou, China, October 2003.

Wagner, S., Truong, P., Vieritz, A. (2003). Response of vetiver grass to extreme nitrogen andphosphorus supply. Proceedings of the Third International Conference on Vetiver and Exhibition, Guangzhou, China

WaterAid Nepal (2006). *Total Sanitation in South Asia: the challenges ahead*, WaterAid

Xia, H. P., Liu S., Ao, H. (2000). Study on Purification and Uptake of Vetiver Grass to Garbage Leachate Proc. Second Intern. Vetiver Conf. Thailand, January

2000...http://picasaweb.google.com/VetiverClients/Vetiver System For Effluent Disposal

Yan-de, J, Zhen-li, H., and Xiao, Y. (2007). Role of soil rhizobacteria in Phytoremediation of heavy metal contaminated soils. J. Zhejiang Univ. Sci., 8(3): 197-207.

Zayed, A., Gowthaman, S., and Terry, N., May-June (1998), Phytoaccumulation of Trace Elements by Wetland Plants: I. Duckweed, Journal of Environmental Quality v 27:3 p 715

Zheng, C., Tu C. and Chen H., (1997). Preliminary study on purification of eutrophic water with vetiver. Proc. International Vetiver Workshop, Fuzhou, China October 1997.

ANNEXES

Annex 1: Plants growth in different treatment ponds with time

Treatment Ponds	Initial Reed Height, (cm)	Weekly Progress Height, (cm)						
		4	8	12	16	20	24	Net Change
Vetiver Pond								
1st row	6	24	43	74	141	170	220	216
2nd row	6	18	40	89	146	185	235	229
3rd row	6	32	60	100	158	198	245	239
4th row	6	16	40	66	125	176	228	222
5th row	6	25	45	80	133	142	229	223
Average	**6±0**	**23± 7.02**	**45.6± 9.24**	**81.8±14.65**	**140.6±13.97**	**174.2±23.17**	**231.4±10.32**	**225.8±9.66**
Common Reed Pond								
1st row	32	50	98	133	145	175	195	160
2nd row	32	58	102	137	156	182	208	176
3rd row	32	62	110	127	140	162	193	163
4th row	32	68	105	133	152	170	198	164
5th row	32	72	101	127	145	170	195	160
Average	**32±0**	**62±9.55**	**103.2±5.05**	**131.4±4.82**	**147.6±7.05**	**171.8±8.18**	**197.8±6.63**	**164.6±7.35**
Mixed Pond								
1st row (Common reed)	32	66	90	135	132	160	168	138
1st row (Vetiver)	6	32	55	90	127	168	200	194
2nd row (Vetiver)	6	28	53	86	114	145	175	169
3rd row (Common reed)	32	35	48	65	109	145	175	150
4th row (Vetiver)	6	18	42	65	75	135	150	144
5th row (Common reed)	32	40	42	66	94	115	140	104
Average	**19±13.64**	**36.5±18.41**	**55±20.38**	**84.5±30.77**	**108.5±24.08**	**144.67±21.22**	**168±23.94**	**149.83±34.36**

Annex 2: Average increment in height

Treatment Ponds	First month	Second month	Third month	Fourth month	Fifth month	Sixth month
vetiver Pond						
1st row	18	19	31	67	29	60
2nd row	12	22	49	57	39	57
3rd row	26	28	40	58	40	47
4th row	10	24	26	59	51	59
5th row	19	20	35	53	35	61
Phragmites Pond						
1st row	15	38	35	12	30	20
2nd row	26	28	35	19	26	26
3rd row	32	35	17	13	22	31
4th row	34	37	28	9	28	28
5th row	37	39	16	10	25	25
Mixed Pond						
1st row (Phragmites)	36	20	33	7	28	8
1st row (Vetiver)	26	23	35	37	41	32
2nd row (Vetiver)	22	25	33	28	31	30
3rd row (Phragmites)	10	13	17	54	36	20
4th row (Vetiver)	12	24	23	30	30	32
5th row (Phragmites)	4	2	24	28	21	25

Annex 3: Change in soil parameters at different time intervals

Duration	Samples	%Org. Matter	%Org. carbon	TN, (%)	P (ppm)
Before Plantation	Vetiver	5.397721	3.130928654	0.24	61.26
	Phragmites	4.3816794	2.541577378	0.20	48.10
	Mixed	5.0167054	2.909921926	0.22	56.33
	Control	5.397721	3.130928654	0.24	61.26
After three months	Vetiver	3.3656378	1.952226102	0.16	34.94
	Phragmites	2.9846222	1.731219374	0.14	30.00
	Mixed	3.6196482	2.099563921	0.17	38.23
	Control	1.905078	1.105033643	0.10	16.01
After six months	Vetiver	0.3810156	0.221006729	0.04	-3.73
	Phragmites	2.6036066	1.510212645	0.13	25.06
	Mixed	1.6510676	0.957695824	0.09	12.72
	Control	2.540104	1.47337819	0.12	24.24

Annex 4: BOD and COD removal at different time interval

Samples	7-Jun, (mg/L)		21-Jun, (mg/L)		7-Jul, (mg/L)		21-Jul, (mg/L)		7-Aug, (mg/L)		21-Aug, (mg/L)	
	BOD	COD	BOD	COD	BOD	COD	BOD	COD	BOD	COD	BOD	COD
Inlet	104.7	853	136.9	960	56.4	869	64.4	960	56.4	693	52.3	859
Vetiver Pond	24.2	267	32.2	192	4.0	176	8.1	192	4.0	133	8.0	192
Phragmites Pond	64.4	608	56.4	533	12.1	507	16.1	533	12.1	448	16.2	565

Samples	7-Jun, (mg/L)		21-Jun, (mg/L)		7-Jul, (mg/L)		21-Jul, (mg/L)		7-Aug, (mg/L)		21-Aug, (mg/L)	
	BOD	COD	BOD	COD	BOD	COD	BOD	COD	BOD	COD	BOD	COD
Mixed Pond	56.4	512	32.2	379	12.1	464	16.1	379	8.1	320	12.1	549
Control Pond	72.5	603	72.5	597	24.2	656	36.2	597	24.2	405	28.2	640

Annex 5: Nitrate for Different Samples

S. N.	Samples	Nitrate, (mg/L)
1.	Inlet	10.21
2.	Vetiver Pond	0.93
3.	Phragmites Pond	1.86
4.	Mixed Pond	1.63
5.	Control Pond	7.15

Annex 6: Total Phosphorous Concentration during Study Period

S. N.	Sampling Ponds	Observed Concentrations, (mg/L)					
		7-Jun	21-Jun	7-Jul	21-Jul	7-Aug	21-Aug
1.	Inlet	29.42	25.74	26.35	29.42	17.16	24.52
2.	Vetiver Pond	10.73	<0.01	<0.01	<0.01	0.61	3.06
3.	Phragmites Pond	22.68	17.16	4.90	18.39	2.45	11.03
4.	Mixed Pond	17.77	1.84	<0.01	4.29	1.84	9.81
5.	Control Pond	35.24	20.84	17.77	17.16	11.03	16.55

Annex 7: Chloride Concentration during Study Period

S. N.	Samples	Observed Concentration, (mg/L)					
		7-Jun	21-Jun	7-Jul	21-Jul	7-Aug	21-Aug
1.	Inlet	51.12	193.6	154	140.8	71	233.2
2.	Vetiver Pond	18.5	61.6	52.8	52.8	11.4	44
3.	Phragmites Pond	35.5	114.4	92.4	110	25.6	110
4.	Mixed Pond	31.2	88	88	88	19.9	92.4
5.	Control Pond	42.6	96.8	83.6	83.6	34.1	171.6

Annex 8: Coliform Count in treatment ponds with time

Samples	7-Jun	21-Jun	7-Jul	21-Jul	7-Aug	21-Aug
Inlet	>500	>500	>500	>300	>300	>300
Vetiver 4^{th} row		>100	>100	87	>100	76
Voutlet	>500	70	93	76	48	33
Phragmites 4^{th} row		>500	>300	>300	>100	>100
Phragmites outlet	>500	>300	>100	95	>100	115
Mixed 1^{st} row		>500	>500	>300	>300	>100
Mixed 4th row		>300	>100	97	72	99
Mixed outlet	>500	>100	>100	82	89	82
Control pond	>500	>300	>300	>300	>100	>100

Annex 9: Questionnaire Checklist

1. What are the total number of Staffs and students in this institution?
   ➢
2. From where do you get the source of water for your institution?
   ➢
3. What is the Daily/ Weekly/ Monthly water Demand of this institution?
   ➢
4. What are the volume of water used for the various purposes?(litre)

Drinking ………………………..	Cooking ………………………..
Toilet …………………………….	Cleaning ………………………..
Washing ………………………….	Laboratory ……………………..

5. Waste Water Dicharge=..............(per day) …………………….(per sec)
6. What is the capacity(in volume litre)
   ➢
7. How do you manage waste water of your institution?
   ➢
8. What Cost do you pay for managing waste water?
   ➢
9. Where do you discharge the Waste water?
   ➢
10. Have you used any treatment system for waste water before discharge?
    ➢
11. General map of the waste water discharge system of the institution:

Annnex 10: General Questionnaire Form

1. What do you say about direct drainage of wastewater into the river systems?

Good ☐ Bad ☐ Doesn't matter anything ☐

2. Have you heard earlier about waste water treatment system or have you seen somewhere before?

Yes ☐ No ☐ Place if seen anywhere ……………………

3. Do you think treatment of wastewater before draining into water resources is important?

Yes ☐ No ☐ Don't Know ☐

4. Did you like this low cost biological waste water treatment system using Vetiver Grass and Reed (Phragmites) in a constructed Wetland? ☐

☐ Don't ☐

5. In case of success of this research, would you like to treat your household wastewater before draining into river or somewhere else?

Yes ☐ No ☐ Not interested ☐

6. If you would like to add your views on it?

………………………………………………………………………………

………………………………………………………………………………

…………………………………………………………….................................

....

<u>Thank you for the help</u> Name: …………………………………….. Address: ………………………………………………

Organization from:

…………………………………………………………………………………

…………………………

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Annex 11: Visitors List

(Biological Waste water Treatment: A comparative study using

Vetiver Grass (*Chrysopogon zizanioides*) and Reed (*Phragmites karka*) in a constructed wetland system

S. No	Age Group				Gender		Name	Address	Organization/ institution from
	< 15	15-30	30-45	> 45	M	F			

Venue: Khwopa College, Dekocha-5, Bhaktapur

Annex 12: List of Photographs

Trimming, mulching and Plant Preservation

Study Plot

Preparation for Exhibition

Working in Laboratory

Sundarijal Public Toilet sewerage treatment

Dhulikhel Reedbed Wastewater Treatment

Visit to different application sites of Wastewater Treatment

Namobuddha Resort Wastewater Treatment

Okharpauwa Landfill Lecheate Treatment

During Plantation (5th March 2014)
Avg Height: Vetiver- 6cm, Narkot- 30cm

After 1 month (5th April 2014)
Avg Height Vetiver- 30cm Narkot- 58

After 2 month (5th May 2014)
Avg Height: Vetiver- 60cm Narkot- 80

After 4 month (5th july 2014)
Avg Height: Vetiver- 120cm Narkot- 128cm

After 5 month (5th August 2014)
Avg Height: Vetiver- 165cm Narkot- 165cm

After 6 month (5th September 2014)
Avg Height: Vetiver- 205cm Narkot- 190cm