1. Introduction

There are various studies in which natural or artificial wetland is used for waste water treatment. A wetland is an area of land whose soil is saturated with moisture either permanently or seasonally, such that it takes on the characteristics of a distinct ecosystem. The capacity for wastewater purification by both natural and artificial wetlands is well documented. Wetlands remove pollutants through a complex variety of biological, physical, and chemical processes. There are many advantages of using constructed wetland over conventional system. The major problem in conventional system is the disposal of secondary pollutants (like Sludge) where as in wetland there is no such problem. The wetland plants need to be harvested regularly to maintain their removal potential. The quality of ground water also improves in the vicinity of any wetland. The use of constructed wetlands (CW) for wastewater treatment has become common practice worldwide. As the application of CW as an advanced treatment, the usual aim is to remove nutrients (phosphorus & nitrogen compounds) which are detrimental to the balance of more sensitive receiving media where it may result in euthrophication. Wetlands are cheaper to construct than any other conventional treatment option. Operation and maintenance cost (energy and supplies) are lower in the case of CW since it is a natural process and it does not require continuous monitoring. The skilled labour is not required for operational stage as well as in monitoring stage. If fluctuation occurs during peak time or season it does not affect the efficiency of the wetland system. No external energy is required during operational stage where as this system will produce biomass which can be use for energy production in separate industry. There are no foul odors or harmful byproducts developed from this system. The plants also do some environmental service by fixation of carbon dioxide and production of oxygen.

Most of the research on constructed wetlands has been conducted in European countries. As a result, the plants most studied for treatment purposes are cattail (*Typha* spp.), bulrush (*Scirpus lacustris*) and reeds (*Phragmitis australis*), as these plants can withstand cold winters. It is known that ornamental plants such as *canna lily* (*Canna flaccida*), *calla lily* (*Z. aethiopica*), elephant ear (*Colocasia sculenta*), ginger lily (*Hedychium coronarium*), and yellow iris (*Iris pseudacorus*) can be used in rock/plant filters to treat septic tank effluents (Wolverton, 1990). Of the floating aquatics, water hyacinth *(Eichhornia crassipes)* and duckweeds (e.g. *Lemna* spp.)

have been studied most extensively for Phosphorus uptake efficiency. However, there is little information about the efficacy of ornamental plants for use in treatment wetlands. The fact that these ornamental plants cannot survive the cold winters in northern countries may be one reason why they have had limited use in constructed wetlands. Since most developing countries are located in tropical and subtropical regions and have limited resources to install conventional wastewater treatment systems, the use of ornamental plants in treatment wetlands should be explored.

Canna lily showed a high promise towards the removal of organic matter and nutrients. The broad flat, alternate leaves, that are such a feature of this plant, grow out from a narrow stem. The leaves are typically solid green. Not many studies are undertaken using this plant and its nutrients (specifically phosphorus) removal efficiency. Keeping this fact in mind the present study will mainly focused on phosphorus removal efficiency of *Canna lily*. In this study the effluent which was supplied to the plants was synthetically prepared in the laboratory. Another important objective of this study is to get an idea about the various fraction of phosphorus present in waste water and the treated water which can be collected from the outlet of the CW system.

Since phosphate removal efficiency will depend upon the plant (*Canna lily*) or more specifically said as phosphate uptake efficiency by the plant, it become important to know the accumulation of phosphorus in various parts of plant tissue. This study will also include this aspect and a brief comparison study has been done to know the effect on accumulation of phosphorus in different part of plant.

Phosphorus has been recognized as the most critical nutrient limiting primary productivity in lakes over long time-scales. It has been shown that the sediment of the lakes can act as an internal source of phosphorus for the overlying water even after the complete control of external point and non-point sources. The stability and chemical form of particulate phosphorus in sediments, in association with the environmental conditions, have been pointed out as the major controlling factors that affect the release of phosphorus from the sediment (Shouliang Huo *et.al,2011)*

Thus, it is necessary to carry out related studies to understand the distribution of different phosphorus fractions in the sediments, and clarify the release or sorption mechanism of phosphorus fractions present in the sediment . For the same reason phosphorus (P) fractionation and its distribution in sediments are also a great concern of this study. Present study will include the relation between the various fraction of phosphorus present in the sediment and their interdependency. This study is design such a way that it will meet the following objectives.

- To study the phosphate removal efficiency by *Canna lily*
- To study the various fractions of phosphate in wastewater, plant, and sediments
- To study the growth of *Canna lily* during this study
- To suggest the efficacy of *Canna lily* for phosphate removal from wastewater

2. Review of Litareture

The capacity of ecosystems that are dominated by aquatic macrophytes to assimilate and decompose input of nutrients and organic matter has resulted in the extended use of such system to treat different type of waste water. Aquatic macrophyte-based waste water treatment systems may be classified into

1) Floating Macrophyte Treatment Systems
2) Submerged Macrophyte Treatment Systems
3) Emergent Macrophyte Treatment Systems
    - Artificial wetland
    - Natural wetland
4) Integrated Macrophyte Treatment Systems

There are various studies of aquatic macrophyte-based wastewater treatment systems in which natural or artificial wetland is used for water treatment. A wetland is an area of land whose soil is saturated with moisture either permanently or seasonally, such that it takes on the characteristics of a distinct ecosystem. Primarily, the factor that distinguishes wetlands from other land forms or water bodies is the characteristic vegetation that is adapted to its unique soil conditions. Wetlands consist primarily of hydro soil, which supports aquatic plants. The water found in wetlands can be saltwater, freshwater, or brackish. Main wetland types include swamps, marshes, bogs and fens. Sub-types include mangrove, carr, pocosin, and varzea. Wetlands cycle both sediments and nutrients balancing terrestrial and aquatic ecosystems. A natural function of wetland vegetation is the up-take and storage of nutrients found in the surrounding soil and water. These nutrients are retained in the system until the plant dies or is harvested by animals or humans. The ability of wetland systems to store nutrients and trap sediment is highly efficient and effective but each system has a threshold. An overabundance of nutrient input from fertilizer run-off, sewage effluent, or non-point pollution will cause eutrophication . The capacity of wetland vegetation to store heavy metals is affected by water flow, number of hectares (acres), climate, and type of plant.

Wetlands remove aquatic pollutants through a complex variety of biological, physical and chemical processes. When plants converts the complex substances into simpler form by enzymatic secretion or any other process like oxidation or reduction it is termed as phytoremediation. Phytoremediation (from Ancient Greek *(phyto)*, meaning "plant", and Latin *remedium*, meaning "restoring balance") describes the treatment of environmental problems through the use of plants that mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere.

2.1 Wetland Vegetation

Wetland treatment system use different water tolerant plant species. The basic type of constructed wetland treatment systems according to flow type include surface flow (SF) wetlands and subsurface flow (SSF) wetlands. There are three broad categories of wetland vegetation.

Emergent Aquatic Macrophytes are plants that live either completely submerged or floating or have some small portion of the plant emerging from the water. They grow within a water table range of 50 cm below the soil surface to 150 cm or more,like- Typha,Canna lily

Floating-leaved aquatic macrophytes includes both species which are rooted in the substrate and species which are free floating on the water surface. i.e. Water hyacinth, Duckweed.

Submerged aquatic macrophytes have their photosynthetic tissue entirely submerged but usually the flower exposed to the atmosphere.

2.1.1 Water hyacinth

General Characteristics

Water hyacinths are free floating aquatic plant, its leaves are broad, thick, glossy. Scientific name of Water hyacinths is ***Eichhorina crassipes***. This type of plant may rise 1 m above the surface of water. Leaves are 10-20 cm across, and float above the water surface. The feathery, freely hanging roots are purple-black.

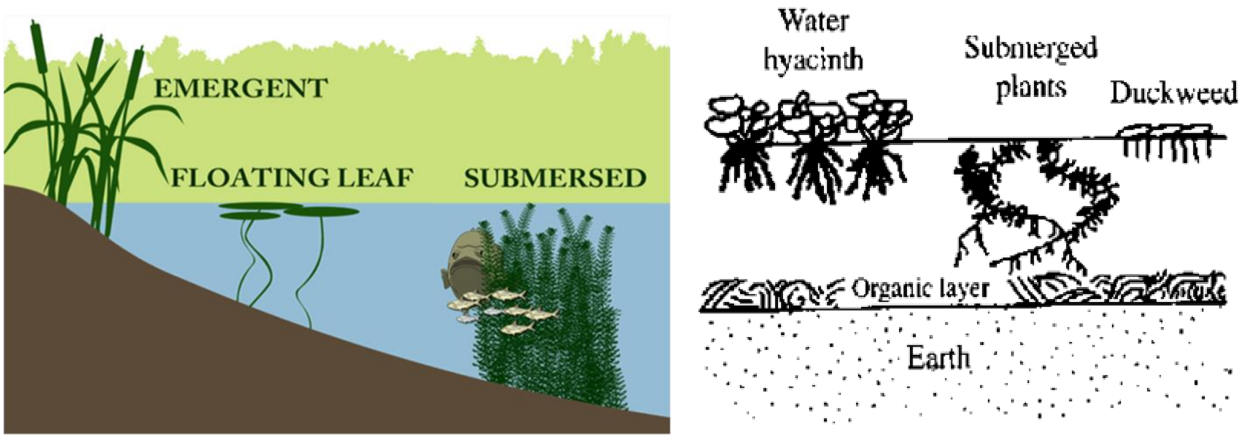

Figure. 2.1 Emergent Aquatic Macrophytes, Floating and Submerged Aquatic Macrophytes

(http://www.google.co.in/imgres?imgurl=http://www.lmvp.org/Waterline/volume14)

Figure. 2.2 Common Wetland Vegetation: 1.Water Hyacinths, 2. *Lemna minor*, 3.*Typha*

(http://www.steingg.com/waterplants.asp, http://www.plantedevis.ro/plante/plante-de-zona-de-inot/lintita-lemna-minor-72, http://www.hlasek.com/typha_laxmanii_a5155.html)

Reproduction

These plants are one of the fastest growing plants and can double there population in two weeks. This type of plants produced large quantities of seeds and they are viable up to thirty years.

2.1.2 *Lemna*

General Characteristics

Lemna is a genus of free floating aquatic plants from Duckweek family. These plants are very rapid growing and are found almost throughout the world. This species are tolerant to a temperature range of 6 to 33˚ C with optimum growth range at 20 to 28˚ C.

Lemna species are small free floating plants. These generally do not exceed 5 cm in length except for *Lemna trisulca* which is elongated and has a branched structure.

There are about 13 species included in Lemna genus. Out of which *Lemna minor* or Common Duckweed is most popular in research level.

Reproduction

Lemna minor reproduces by vegetative reproduction. Leaves of the frond grow to their maximum size and then they detach themselves from the parent frond and developed its own root thus forming a new frond.

2.1.3 *Typha*

General Characteristics

Typha is a genus of about eleven species of monocotyledonous flowering plants in the family Typhaceae. The genus has largely Northern Hemisphere distribution, being found in Varity of wetland habitats. This plant has many common names like in British English as bulrush, in American English as cattail, punks in Australia as cumbungi.

Reproduction

Typha leaves are alternate and mostly basal to a simple, joint less stem that eventually bears the flowering spikes. Typha plants monoecious and bear unisexual, wind-pollinated flowers.

2.1.4 *Canna lily*

Canna (or canna lily, although not a true lily) is a genus of nineteen species of flowering plants. The closest living relations to cannas are the other plant families of the order Zingiberales, that is the Zingiberaceae (gingers), Musaceae (bananas), Marantaceae, Heliconiaceae, Strelitziaceae, etc. *Canna* is the only genus in the family Cannaceae. Such a family has almost universally been recognized by taxonomists. The APG II system of 2003 (unchanged from the APG system, 1998) also recognizes the family, and assigns it to the order Zingiberales in the clade commelinids, in the monocots.

The species have large, attractive foliage and horticulturists have turned it into a large-flowered and bright garden plant. In addition, it is one of the world's richest starch sources, and is an agricultural plant.

Although a plant of the tropics, most cultivars have been developed in temperate climates and are easy to grow in most countries of the world as long as they can enjoy at least 6–8 hours average sunlight during the summer, and are moved to a warm location for the winter. See the *Canna* cultivar gallery for photographs of *Canna* cultivars.

General Characteristics

The plants are large tropical and subtropical perennial herbs with a rhizomatous rootstock. The broad, flat, alternate leaves, that are such a feature of this plant, grow out of a stem in a long narrow roll and then unfurl. The leaves are typically solid green but some cultivars have brownish, maroon, or even variegated leaves.

The flowers are composed of three sepals and three petals that are seldom noticed by people, they are small and hidden under extravagant stamens. What appear to be petals are the highly modified stamens or staminodes

Figure. 2.3 ***Canna lily***

The flowers are typically red, orange, or yellow or any combination of those colors, and are aggregated in inflorescences that are spikes or panicles (thyrsus). Although gardeners enjoy these odd flowers, nature really intended them to attract pollinators collecting nectar and pollen, such as bees, hummingbirds and bats. The pollination mechanism is conspicuously specialized. Pollen is shed on the style while still in the bud, and in the species and early hybrids some is also found on the stigma because of the high position of the anther, which means that they are selfpollinating. Later cultivars have a lower anther, and rely on pollinators alighting on the labellum and touching first the terminal stigma, and then the pollen.

The wild species often grow to at least 2–3 m (6.6–9.8 ft) in height but there is a wide variation in size among cultivated plants; numerous cultivars have been selected for smaller stature. Cannas grow from swollen underground stems, correctly known as rhizomes, which store starch, and this is the main attraction of the plant to agriculture, having the largest starch particles of all plant life.

Canna is the only member of the Liliopsida Class (monocot group) in which hibernation of seed is known to occur, due to its hard, impenetrable seed covering. Cannas grow best in full sun with moderate water in well drained rich or sandy soil. In areas which go below about -10˚C in winter, the ribosome's can be dug up before freezing and stored in a protected area(above 7˚C).

Location is important. Canna lily should be planted in locations away from hot walls or reflected light but in full sun or very nearly so. In our climate they also do well under very light shade, but not at all in deep shade.

2.2 Contaminant Removal mechanisms in the Constructed Wetlands

Wetland can effectively remove or convert large quantities of pollutants from point sources (municipal, industrial and agricultural wastewater) and non-point sources (mines, agriculture and urban runoff), including organic matter, suspended solids, trace metals and nutrients. The focus on wastewater treatment by constructed wetlands is to optimize the contact of microbial species with substrate, the final objective being the bioconversion to carbon dioxide, biomass and water. Wetlands are characterized by a range of properties that make them attractive for managing pollutants in water (Bavor & Adcock, 1994). These properties include high plant productivity, large adsorptive capacity of the sediments, high rates of oxidation by micro flora associated with plant biomass, and a large buffering capacity for nutrients and pollutants.

1. **Physical processes**: Sedimentation and filtration are the main physical processes leading to the removal of wastewater pollutants. The effectiveness of all processes (biological, chemical, physical) varies with the water residence time (i.e., the length of time the water stays in the wetland). Longer retention times accelerate the remove of more contaminants, although too-long retention times can have detrimental effects.

2. **Chemical processes:** Metals can precipitate from the water column as insoluble compounds. Exposure to light and atmospheric gases can break down organic pesticides, or kill disease producing organisms (EPA, 1995). The pH of water and soils in wetlands exerts a strong influence on the direction of many reactions and processes, including biological transformation, partitioning of ionized and un-ionized forms of acids and bases, cation exchange, solid and gases solubility.

3. **Biological processes**: There are six major biological reactions involved in the performance of constructed wetlands, including photosynthesis, respiration, fermentation, nitrification, de-nitrification and microbial phosphorus removal (Mitchell, 1996). Photosynthesis is performed by wetland plants and algae, with the process adding carbon and oxygen to the wetland. Both carbon and oxygen drive the nitrification process. Plants transfer oxygen to their roots, where it passes to the root zones (rhizosphere). Respiration is the oxidation of organic carbon, and is performed by all living organisms, leading to the formation of carbon dioxide and water. The common microorganisms in the CW are bacteria, fungi, algae and protozoa. The maintenance of optimal conditions in the system is required for the proper functioning of wetland organisms.

2.2.1 Removal mechanism of Organic compounds

Settle able organics are rapidly removed by wetland systems under low flow conditions by deposition and filtration. Attached and suspended microbial growth is responsible for removal of soluble organics. Organic compounds are degraded aerobically or anaerobically. The oxygen required for aerobic disintegration is supplied directly from the atmosphere by diffusion or oxygen leakage from the macrophytes roots into the rhizosphere. Uptake of organic matter by the macrophytes is negligible compare to biological degradation (Watson et.al 1989).

Anaerobic degradation of soluble organic matter is governed by aerobic heterotrophic bacteria. The autotrophic group of bacteria which degrades organic compound containing nitrogen under aerobic condition is called nitrifying bacteria. Cooper et.al (1996) pointed out that both group consumes organic but the faster metabolic rate of heterotrophs means that they are mainly responsible for the reduction of BOD of the system.

2.2.2 Removal mechanism of Suspended Solids

In case of suspended solids removal process sedimentation and filtration will play the major role. Whereas microbial growth in the wetland system helps in removal of colloidal solids. When the growth of vegetation in a particular wetland system is more the removal efficiency of suspended solids and colloidal solids is more.

2.2.3 Removal mechanism of Heavy Metals / Contaminants

Constructed wetlands are wastewater treatment systems that combine biological, chemical, and physical treatment mechanisms for water quality improvement (Crites 1992). The mechanisms for water quality improvement in wetlands include adsorption, complexation, chemical precipitation, and plant uptake (Reed et.al. 1995).

2.2.4 Removal mechanism of Nutrients (Nitrogen and Phosphorus)

Nitrogen: Nitrogen processes in wetland soils include: Nitrification (in aerobic zones), de-nitrification (in aerobic zones) – releasing N_2 and N_2O gases, plant uptake, sedimentation, decomposition, ammonia volatilization and accretion/accumulation of organic N in peat because of redox potential of hydric sediment.

Phosphorus: The fate of phosphorus is quite different in wetland soils, since there is no mechanism comparable to de-nitrification as phosphorus has no gaseous phase. Consequently although the processes of plant uptake, sorption, decomposition and long-term storage occur, phosphorus tends to accumulate in wetlands at a higher rate than does nitrogen. Precipitation of phosphate minerals can provide a significant sink for phosphorus in wetlands with large stores or inputs of iron and aluminum (low pH wetlands) or calcium (high-pH wetlands). Although wetlands may remove and store substantial quantities of phosphorus, they also potentially release a significant amount of phosphorus to downstream ecosystems. It's estimated that the long-term elimination rate of the Phosphorus through plants is about 0.05g/m^2/day in a constructed wetland.(Kapanen, 2008; Kim et.al,2002)

2.3 Role of aquatic mycrophytes in secondary-treated wastewater

Various wetland vegetation like- water hycinths(***Eichhorina crassipes***), common duckweed(***Lemna minor***), cattail(***Typha***), ***Canna lily*** have shown a good result in various studies.

2.3.1 BOD, COD, TDS, TSS Removal Efficiency

The study constructed by Shah *et.al*. (2010), potential of Water Hyachinth (*Eichhorina crassipes*) is treating dye waste water was studied. It was found that the plant was able to remove TDS by 35%, BOD by 42% and COD by 35% for 25% dilution of waste water. For higher dilutions the plant was not able to survive.

In another study done by R. M. Gersberg *et.al.*(1986) describes investigations using artificial wetlands which quantitatively assess the role of each of three higher aquatic plant types, *Scirpus validus* (bulrush), *Phragmites communis* (common reed) and *Typha latifola* (cattail), in the removal of nitrogen (via sequential nitriflcation-denitrification), BOD and TSS from primary municipal wastewaters. During the period August 1983-December 1984, with a mean effluent BOD concentration of 5.2 ± 5.3mg/l in the primary wastewater inflow (hydraulic application rate = 4.7 cm /day). Mean BOD removal efficiencies (relative to the inflow) were 96, 81, 74 and 69%, for the bulrushes, reeds, cattails and unvegetated beds, respectively. The mean effluent value of 22.3 mg/l for the reed bed was also significantly lower than the value for the unvegetated bed. The cattail bed was again the poorest performer with regard to BOD removal, having a mean effluent level not significantly different from the unvegetated bed. Only the reed and bulrush beds showed treatment equal or better than secondary treatment quality (30 mg/l). Since BOD removal (organic carbon compound degradation) is enhanced under aerobic conditions, it is reasonable to assume that the superior treatment afforded by the bulrush (and to a lesser extent the reeds) was due to plant translocation of oxygen to an otherwise anaerobic zone, thereby stimulating breakdown of carbonaceous compounds. As for removal of total suspended solids, the mean effluent levels for all wetland beds were low. The fact that the mean values for the vegetated beds were not significantly different from the un vegetated bed indicates that removal of suspended solids is almost due entirely to physical processes (sedimentation and filtration) rather than biological processes associated with the microbial community or with the higher plants.

2.3.2 Removal of Heavy Metal

The study conducted by Snyder (2006), Water Hyacinth is used to remove the arsenic from drinking groundwater of Bangladesh. He has found that the plant can remove 300 ppb of arsenic down to 10 ppb.

In another study removal of heavy metals by *Lemna minor* (common duckweed) has been studied by Axtell et.al.(2003). They have found that this plant has a overall efficiency of 76% in lead removal. The average value of removal by *Lemna minor* was 69% at low level of lead and 83% at high concentration level. In the same study Nickel concentration reduced by 82%.Within a span of 4 days, the plant was capable of removing about 75-85% of Cd from 100L of waste water containing 3.0-7.0 mg/l of metal at an optimum pH of 6.5.

2.3.3 Removal of Total Nitrogen

A study done by R. M. Gersberg *et.al.*(1986) describes investigations using artificial wetlands which quantitatively assess the role of each of three higher aquatic plant types, *Scirpus validus* (bulrush), *Phragmites communis* (common reed) and *Typha latifola* (cattail), in the removal of nitrogen (via sequential nitriflcation-denitrification) from primary municipal wastewaters. During the period August 1983-December 1984, with a mean ammonia concentration of 24.7mg/l in the primary wastewater inflow (hydraulic application rate = 4.7 cm/day) was reduced to mean effluent levels of 1.4 mg/lfor the bulrush bed, 5.3 mg/l for the reed bed and 17.7mg/l for the cattail bed, as compared to a mean value of 22.1 mg/l for the unvegetated (control) bed. For all three vegetated beds, the mean effluent ammonia values were significantly below that for the unvegetated bed and for the inflow. The bulrushes and reeds (in that order) proved to be superior at removing ammonia, both with mean effluent levels significantly below that for the cattail bed. The high ammonia-N (and total N) removal efficiencies shown by the bulrush and reed beds are attributed to the ability of these plants to translocate 02 from the shoots to the roots. The oxidized rhizosphere so formed stimulates sequential nitrification-denitrification

In another study done by Chris C. Tanne et.al (1994) the effect of influent loading rate on mass removal of nitrogen and phosphorus from dairy parlor wastewaters was compared in four pairs of planted *(Schoenoplectus validus)* and unplanted gravel-bed wetlands (each 19 m2). The wetlands were operated at nominal retention times of 7, 5.5, 3 and 2 days, with in and outflows sampled fortnightly over a 20 month period. Hydraulic flows were monitored to enable calculation of the mass flows of nutrients, and plant biomass and tissue nutrient levels sampled to evaluate plant nutrient uptake. As theoretical wastewater retention times increased from 2 to 7 days, mean reduction of TN increased from 12 to 41% and 48 to 75% in the unplanted wetlands and planted wetlands, respectively.

2.3.4 Removal of Phosphate (P)

In the study conducted by DeBusk et.al.(1995),10 emergent species were studied for the removal of dairy wastewater. The species studied were cattail *(Typha domingensis);* pickerelweed *(Pontederia cordata);* canna lily *(Canna flaccida);* Utongue *(Sagittaria lancifolia);* arrowhead *(Sagittaria latifolia);*lizard's tail *(Saururus cernuus);* green arum *(Peltandra virginica);* giant reed *(Phragmites australis);* soft rush *(Juncus effusus);* and bulrush *(Scirpus validus).* Most species exhibited increased tissue P contents and greater biomass yields when supplemental P fertilization was provided. Canna lily and pickerelweed were the two species that provided highest rates of foliage P uptake from the enriched wastewater (uptake rates of 173 and 66 mgP/m2-day, respectively). Shoot: Root biomass ratios for canna lily and pickerelweed were 1.2 and 0.7, respectively, so "whole-plant" P uptake rates were likely twice the values shown above. Several studies were conducted with the terrestrial crops alemangrass *(Echinochloa polystachia),* paragrass *(Brachiaria mutica),* floralta limprograss (Hemarthria *altissima)* and bermudagrass *(Cynodon dacoty).* Effects of primary lagoon effluent application rates (4.5 vs. 8.9 cm/week) and water depth on biomass production and P uptake were evaluated. From February to May, P uptake by bermudagrass, paragrass, alemangrass and floralta limpograss cultured at a 4.5 cm/wk application rate (with drainage of the [eachate 24 h after effluent application) was 34, 29, 28 and 16 mgP/m^2-day, respectively. Phosphorus uptake by alemangrass (22 mgP/m^2-day) and paragrass (18 mgP/m^2-day)was reduced by cultivation in 9 cm of standing water. Phosphorus uptake (based on foliage harvests) by floralta limpograss (41 mgP/m^2-day) and alemangrass (49 mgP/m^2-day) was enhanced by cultivation at a 8.9 cm/week application rate, due both to increased biomass production and increased tissue P concentrations.

In another study conducted by Gaoa *et.al* (2009), 5 mycrophytes were studied for phosphorus removal from water of eutrophic lake. Five submerged macrophytes, *Ceratophyllum demersum*, *Elodea canadensis*, *Potamogeton crispus*, *Myriophyllum spicatum* and *Vallisneria spiralis* were selected and their relative growth rate (RGR) and the capacity of removing phosphorus in greenhouse were evaluated by hydrotropic experiments of two seasons (spring and autumn). The results showed that the RGR of *C. demersum* was the highest (1.29 for spring and 0.58 for autumn) among the five macrophytes, while that of *P. crispus* was the lowest (0.039) in the spring experiment and *M. spicatum* was the lowest (0.022) in the autumn experiment. Also, total phosphorus (TP) removal rates of *C. demersum* (91.75% and 92.44%) during the spring and autumn were the highest in the five macrophytes. Among the five macrophytes, the order of phosphorus removal capacities was: *C. demersum* > *E. canadensis* > *P. crispu* during the spring. But for autumn, the order was *C. demersum* > *V. spiralis* > *M. spicatum*.

2.4 Mobility of Phosphorus in the Sediment of Wetland System

Various pollutants may be adsorbed to the sediments accumulated on the bottom of rivers or lakes. These sediments may accumulate over long periods and can act as new pollutant sources to the overlying water many years after the water quality has improved (Lijklema et al., 1993; Masunaga et al., 1993; Abrams and Jarrell, 1995). Pollutants such as phosphorus may be transferred from the water column to sediment layer with biochemical and physical reactions such as ion exchange, adsorption, and precipitation (Stumm and Morgan, 1996).The pollutants may be released from the sediments if the overlying water quality changes (Fukushima et al., 1984; Furumai et al., 1989). Mechanisms for reintroduction include advection, ion exchange, molecular diffusion, and biologically mediated changes. The characteristics of sediments, as well as overlying water quality, will affect the release rate.

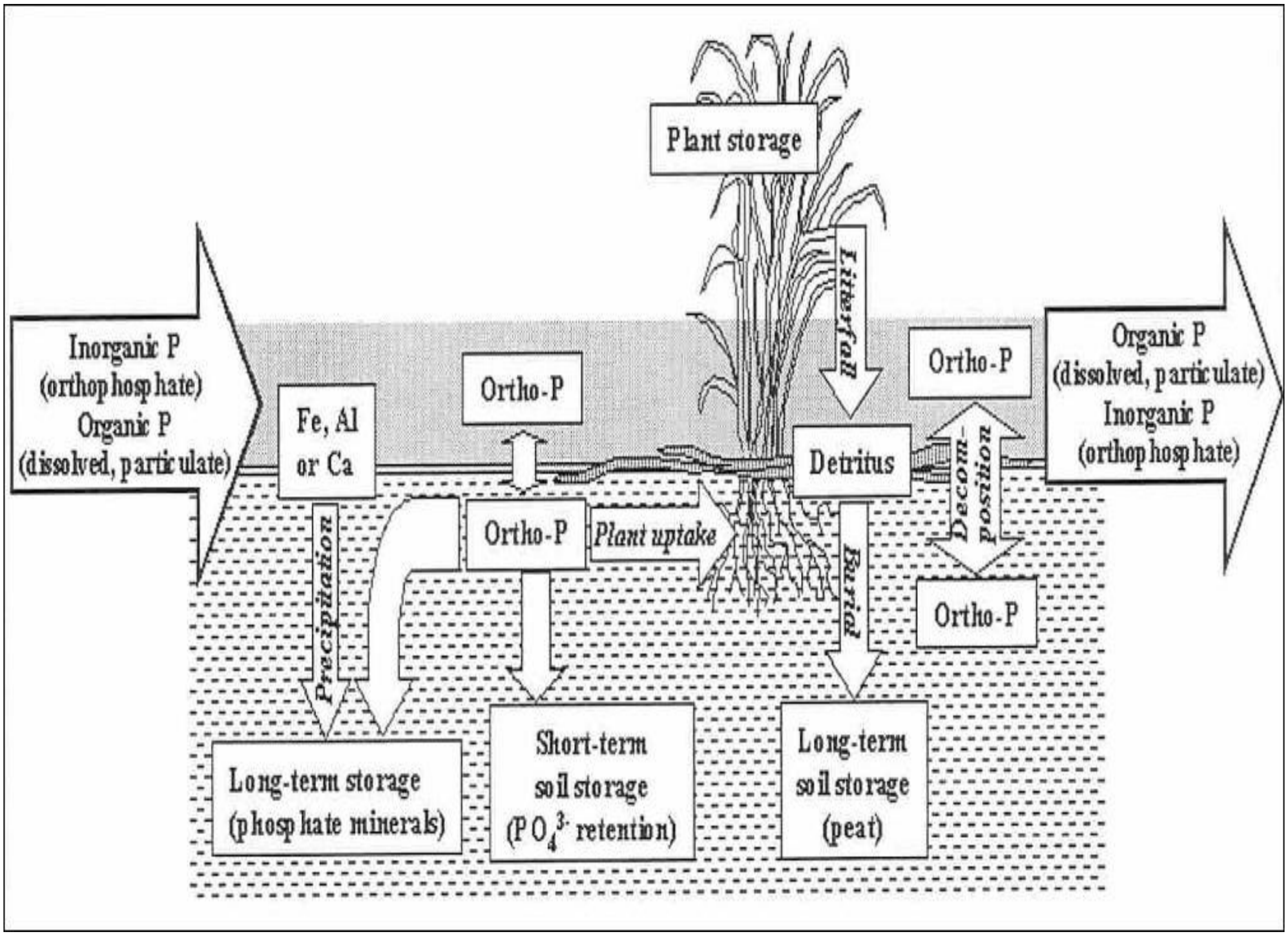

Figure. 2.4 Diagram courtesy of Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

2.4.1 Phosphorus Fraction Present in Sediments

Eutrophication is a common problem in any aquatic system. To assess the risk of eutrophication in aquatic systems it is necessary to know not only the total phosphorus content in the sediments but also its distribution among the different sediment phases. For this purpose, chemical fractionation methods using extracting agents have been widely applied . These methods attempt to differentiate the sediment phosphorus pool in the following fractions: labile, associated to Al, Fe and Mn oxides and hydroxides, associated to Ca, organic and residual . Several compounds are included in the organic fraction: sugar phosphates, nucleotides, humic and fulvic substances, phosphate esters, phosphonates.Although some authors have proposed methods for the fractionation of organic phosphorus in sediments, most of them consider the organic phosphorus in one single fraction, due to the difficulty of separation and identification of these compounds. Most procedures attempt to separate at least five of the fractions mentioned non-apatite inorganic phosphorus (NAIP), bound to Al, Fe and Mn oxyhydrates; apatite phosphorus (AP), bound to Ca; inorganic phosphorus (IP); organic phosphorus (OP) and total phosphorus (TP).

The relationship between these five types of phosphorus-

$$IP = AP + NAIP$$

$$OP = TP - IP$$

In a study done by Bin Zhang *et.al* (2011) the phosphorus fractions and phosphate sorption and release characteristics of the surface soil in the water-level-fluctuating zone were investigated. The results indicated that the total phosphorus (TP) contents in the soil varied from 0.39mg/g to 0.81mg/g; inorganic phosphorus (IP) was the major fraction of the TP. Calcium bound phosphorus (Ca-P) was the main fraction of IP. For all the soil samples, the rank order of P-fraction was Ca-P> Organic P> Phosphorus bound to Al, Fe and Mn oxides. Phosphorus sorption in the sediments occurred within 2h and reached to equilibrium in 12h.

In another study done by J. D. H. Williams *et.al* (1980), P uptake by the algae was related to the amount of nonapatite inorganic phosphorus in the sediments. Apatite phosphorus was not used, and the bluff samples, in which over 90% of total P was in this form, did not support algal growth. The nonapatite inorganic P fraction was highly correlated with the amounts of inorganic phosphorus extracted by three standard techniques for estimating “available P” (extraction by NaOH and nitrilotriacetic acid solutions and by H-resin) and cell uptake equaled NaOH-extractable inorganic P in several instances. Uptake of P by the cells varied from 8 to 50% of total P and from 38 to 83% of nonapatite inorganic P when measured directly. Organic phosphorus in the sediments was not utilized by the algae. Percentage utilization of total P was in general highest when total P concentration in the sediments was itself high.

3. Materials and Methods

3.1 Bench-scale wetland

A bench-scale subsurface flow wetland (SSFW) was built in laboratory at Delhi Technological University, Bawana road, New-Delhi. The system consisted of one cell as working condition and another one left in stand by condition; 78.5 cm wide and 106 cm long. The substrate consisted of a 33.5-cm layer of crushed rock (3–5 cm diameter).The porosity of the media was $\varphi = 0.161$. The water level was kept at 5 cm above the gravel surface and the volume of water is to apply daily was 45 L. *Canna lily* was planted in the cell of dimension 110cm×80cm×45cm, the total volume involved in the experiment was approximately 400 L.

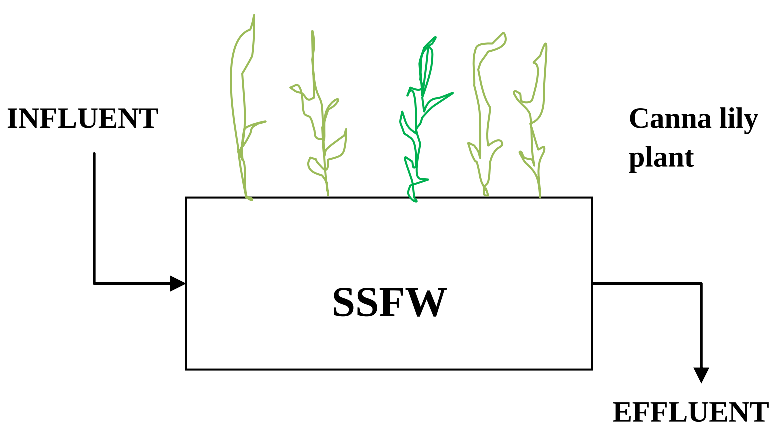

Figure. 3.1 Line Diagram of Experimental plot planted with Canna lily

3.2 Plants

Canna lily cell was planted with 92 plants (stem count), on 15 cm centers (distance between them) with a plant density of 110.56 unit/m^2. The plants were planted before for separate purpose and it was around 43.18 cm tall when this present experiment was started. The system was maintained at a temperature range of 18-27◦C and 13-14 h of natural light per day.

After the experimental period total stem count was 120 and the height of the plant varies between 157.48 ±2.79 cm.

3.3 Experiments

During the experiment the flow rate had been taken constant and HRT (Hydraulic Retention Time) was 1 day on wetland .The water, fed to the plant had phosphorus concentration of **5 ppm,** was synthetically prepared in the laboratory. It was generally found that the concentration of phosphorus concentration in the waste water varies between **4.1-6.8 ppm.**

3.3.1 Experiment-I (Waste Water Analysis)

Samples of treated water were collected after every 24 hours (HRT-1 day) for 30 days. The samples were tested for the fraction of Phosphate concentration in Available-Phosphate (AP) form and in Total-Phosphate (TP) from using standard method as prescribed by APHA. (1997).

3.3.2 Experiment-II (Sediment Analysis)

Soil/Sediment samples were collected before starting and after completion of total experimental period. Phosphate fractions i.e. Ca-bound phosphate (AIP), Fe, Mn, Al-bound phosphate (NAIP) ,Inorganic Phosphate(IP), Organic Phosphate(OP) present in these samples had been determined by the standard method.

3.3.2 Experiment-III (Plant Analysis)

Various parts of plant tissues were collected to determined the Total Phosphate (TP) concentration present in the those parts of plant and a comparison study had been conducted before and after the experimental period.

Figure. 3.2 Experimental plot filled with gravel-sand bed planted with Canna lily

Figure. 3.3 Sample collection from the constructed SSFW

3.4 Phosphate-Fractions

3.4.1 Available Phosphate

Available Phosphate was measured using Ammonium molybdate -Stannous chloride method.

Preparation of chemicals required

1) **Ammonium molybdate**

25 gm of ammonium molybdate was dissolved in 175 ml of distilled water. In a separate conical flask add 280 ml of concentrated sulphuric acid in 400 ml of distilled water.mix the two solution and make the final volume 1 l.

2) **Stannous chloride**

2.5 gm of stannous chloride was dissolved in 100 ml of Glycerol. Heat the solution with intermittent mixing.

3) **Preparation of standards**

0.143 gm of potassium dihydrogen phosphate was mixed in 1l of distilled water to obtain 100 ppm of phosphate stock standard. 10 ml of this solution was taken and final volume was made 100 ml to obtain 10 ppm of phosphate standard.

Serial dilution was used than to prepare 10 standards in the range of 0.1 to 1.0 ppm.

Preparation of Standard curve

1) To 50 ml of 0.1 ppm phosphate standard add 2 ml of ammonium molybdate and mix it well.
2) Add 5 drops of stannous chloride to it and mix it well.
3) Let it stand for 5 min, blue colour appears.
4) Note the absorbance at 690 nm on spectrophotometer.
5) Repeat the above step for 0.2ppm, 0.3ppm, 0.4ppm, and 1ppm.

6) Plot the graph between concentration and absorbation.Best fit line was drawn.

Measurement of phosphate

1) To 50 ml of sample add 2 ml of ammonium molybdate and mix it well.
2) Add 5 drops of stannous chloride to it and mix it well.
3) Let it stand for 5 min, blue colour appears.
4) Note the absorbance at 690 nm on spectrophotometer.
5) Multiplication of absorbance with graph factor gives the phosphate concentration of sample.

Figure. 3.4 Standards of Phosphate

3.4.2 Total Phosphate

Total Phosphate is measured by Sulfuric acid-Nitric acid digestion method. In this study this fraction of phosphate is determined for both water and sediment samples. The whole procedure remain same for both the cases, the difference is in case of sediment sample after acid digestion we have to centrifuge the sample to get a clear extract.

Chemicals Required

1) Concentrated Sulfuric Acid (H_2SO_4)
2) Concentrated Nitric Acid (HNO_3)

Apparatus Required

A Microwave with at least 6 digestive units

Measurement of Total Phosphate

1) To 20 ml of sample adds 0.4 ml of conc. H_2SO_4 and add 2ml of conc. HNO_3.
2) Keep this solution in the digestion unit and set the microwave for 5-10 minute at low (200KW) level.
3) After completion of digestion add NaOH solution to neutralize the solution.
4) To 50 ml of sample add 2 ml of ammonium molybdate and mix it well.
5) Add 5 drops of stannous chloride to it and mix it well.
6) Let it stand for 5 min, blue colour appears.
7) Note the absorbance at 690 nm on spectrophotometer.
8) Multiplication of absorbance with graph factor gives the phosphate concentration of sample.

Figure. 3.5 Extraction of Total Phosphate in Microwave Digestion Unit

3.4.3 Inorganic Phosphate

To know the amount of Inorganic Phosphate present in sample Hydrochloric Acid method is used. In this study inorganic phosphate is only determined for sediment samples.

Chemicals Required

1) 1.0 M Hydrochloric Acid

Measurement of Inorganic Phosphate

1) To the 100 mg of sample adds 20 ml of 1.0 M conc. HCl.
2) Shaking for 2 hour at 150 r.p.m.
3) To get a clear extract centrifuge the sample and collected the clear extract.
4) To 50 ml of extract add 2 ml of ammonium molybdate and mix it well.
5) Add 5 drops of stannous chloride to it and mix it well.
6) Let it stand for 5 min, blue colour appears.
7) Note the absorbance at 690 nm on spectrophotometer.
8) Multiplication of absorbance with graph factor gives the phosphate concentration of sample.

Figure. 3.6 Photograph of Sediment collected from the wetland

3.4.4 Non-Appetite Inorganic Phosphate (NAIP)

This is a fraction of inorganic phosphate which is bound to Fe, Mn, Al. To know the amount of NAIP present in a sediment sample Sodium Hydroxide method is followed.

Chemical required

1) 1.0 M NaOH solution.

Measurement of Non-Appetite Inorganic Phosphate

1) To 100 mg of soil sample add 20 ml of 1.0 M NaOH solution.
2) Shaking for 2 hour at 150 r.p.m.
3) To get a clear extract centrifuge the sample and collected the clear extract.
4) To 50 ml of extract add 2 ml of ammonium molybdate and mix it well.
5) Add 5 drops of stannous chloride to it and mix it well.
6) Let it stand for 5 min, blue colour appears.
7) Note the absorbance at 690 nm on spectrophotometer.
8) Multiplication of absorbance with graph factor gives the phosphate concentration of sample.

3.4.5 Appetite Inorganic Phosphate (AIP)

This is a fraction of inorganic phosphate which is bound to Ca. To know the amount of AP present in a sediment sample Hydrochloric Acid method is followed.

Chemical required

1) 1.0 M HCl solution.

Measurement of Non-Appetite Inorganic Phosphate

1) Collect the residue which was left in NAIP measurement during centrifuge.

2) To the sample add 20 ml of 1.0 M HCl solution.

3) Shaking for 2 hour at 150 r.p.m.
4) 4.To get a clear extract centrifuge the sample and collected the clear extract.
5) 5.To 50 ml of extract add 2 ml of ammonium molybdate and mix it well.
6) Add 5 drops of stannous chloride to it and mix it well.
7) Let it stand for 5 min, blue colour appears.
8) Note the absorbance at 690 nm on spectrophotometer.
9) Multiplication of absorbance with graph factor gives the phosphate concentration of sample.

3.5 Measurement of Phosphate in Plant Tissue

To know the concentration of phosphate present in plant tissue various sample of plant has been collected like- flower, leaves, stem, root. Before measurement of phosphate concentration, this sample should goes through some sample preparation process.

Sample Preparetion

1) Collect the various parts and left them for sun drying.
2) When enough drying was done kept these within a oven for 1 day not more than 60˚C.
3) Crush them by hand or mechanically and made a powered form of these sample and collect them in separate container.

Measurement of Phosphate

1) To 100 mg of sample adds 0.4 ml of conc. H_2SO_4 and add 2ml of conc. HNO_3.
2) Keep this solution in the digestion unit and set the microwave for 5-10 minute at low(200kw) level.
3) After completion of digestion add NaOH solution to neutralize the solution.
4) To 50 ml of sample add 2 ml of ammonium molybdate and mix it well.

Figure. 3.7 Photograph of various parts of plant

5) Add 5 drops of stannous chloride to it and mix it well.

6) Let it stand for 5 min, blue colour appears.

Measurement of Phosphate

1) To 100 mg of sample adds 0.4 ml of conc. H_2SO_4 and add 2ml of conc. HNO_3.

2) Keep this solution in the digestion unit and set the microwave for 5-10 minute at low (200KW) level.

3) After completion of digestion add NaOH solution to neutralize the solution.

4) To 50 ml of sample add 2 ml of ammonium molybdate and mix it well.

5) Add 5 drops of stannous chloride to it and mix it well.

6) Let it stand for 5 min, blue colour appears.

7) Note the absorbance at 690 nm on spectrophotometer.

8) Multiplication of absorbance with graph factor gives the phosphate concentration of sample.

1. Results and Discussion

This study was mainly concentrated on the Phosphorus uptake efficiency of *Canna lily* and the effect on *Canna lily* due to application of waste water which is synthetically prepared in the laboratory.

4.1 Ambient temperature profile

Ambient temperature has been studied during the experimental period to know the effect of temperature on the plant *Canna lily*. The minimum and maximum temperature of the day has noted down daily, from 2nd April`13 to 18$^{th}$ May`13. The average sun shine hour in a day varies between 13 h to 14 h. The average maximum temperature during the study was 36.67˚C and minimum average temperature was 21.36˚C.

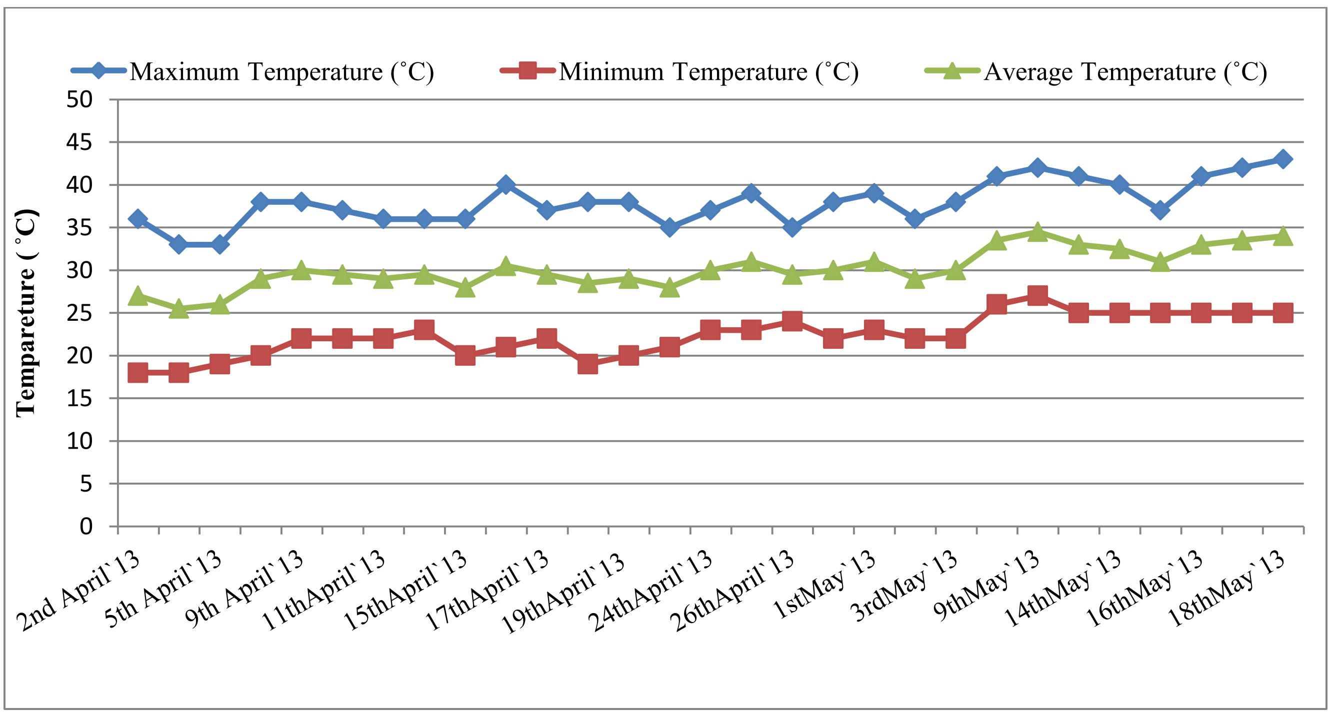

Figure . 4.1 Ambient temperature profile

Table 4.1 Ambient temperature profile during the study period

(file:///J:/project/MAJOR%20PROJECT/TEMP-APRIL, file:///J:/project/MAJOR%20PROJECT/TEMP-MAY`13)

Date	Maximum Temperature (˚C)	Minimum Temperature (˚C)	Average Temperature (˚C)
2nd April`13	36	18	27.00
4th April`13	33	18	25.50
5th April`13	33	19	26.00
8th April`13	38	20	29.00
9th April`13	38	22	30.00
10th April`13	37	22	29.50
11th April`13	36	22	29.00
12th April`13	36	23	29.50
15th April`13	36	20	28.00
16th April`13	40	21	30.5.0
17th April`13	37	22	29.50
18th April`13	38	19	28.50
19th April`13	38	20	29.00
23rd April`13	35	21	28.00
24th April`13	37	23	30.00
25th April`13	39	23	31.00
26th April`13	35	24	29.50
27th April`13	38	22	30.00
1st May`13	39	23	31.00
2nd May`13	36	22	29.00
3rd May`13	38	22	30.00
6th May`13	41	26	33.50
9th May`13	42	27	34.50
10th May`13	41	25	33.00
14th May`13	40	25	32.50
15th May`13	37	25	31.00
16th May`13	41	25	33.00
17th May`13	42	25	33.50
18th May`13	43	25	34.00
Range	33-43	18-27	25.50-34.00
Mean	36.67	21.63	29.15

4.2 Analysis of Waste Water Samples

Phosphorus is one of the major pollutant/nutrient present in waste water. The average concentration of phosphorus observed in municipal raw sewage is of in the order of 10 to 15 mg/l. Where in most of the cases after secondary treatment the concentration was found to be in the range of 4.8-6.2 mg/l. The nutrients removal was adopted as tertiary treatment to the waste water. In this study waste water was synthetically prepared in the laboratory and the study was conducted for a period of 30 days. Waste water was applied everyday during this study periods and samples were collected from the outlet every 24 hr Hydraulic Retention Time (HRT).

Removal of Total Phosphate (TP)

The influent concentration in the present study was varying between **3.12-8.43 mg/l** and the concentration in effluent varied from **0.42-3.27 mg/l** with an average value of **1.77 mg/l**. The major removal mechanism of Total Phosphorus (TP) was filtration, sedimentation, and absorption of phosphate by the whole wetland system.

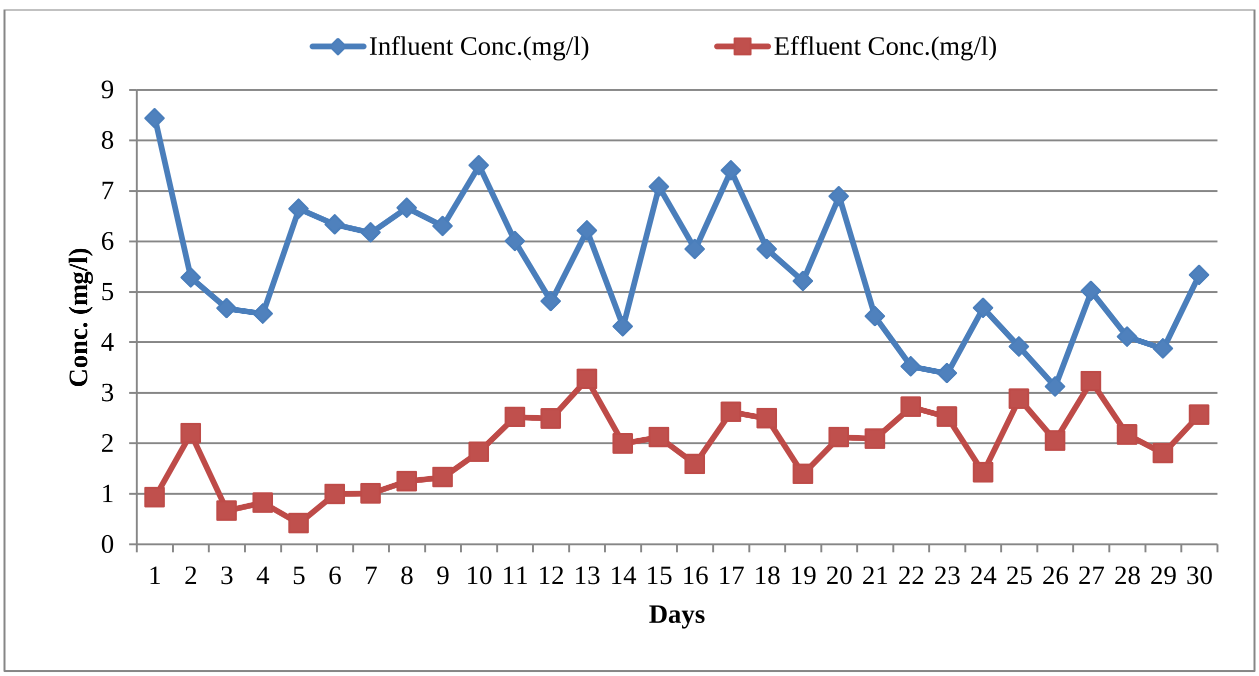

Figure. 4.2 Influent and Effluent concentration of TP in ppm

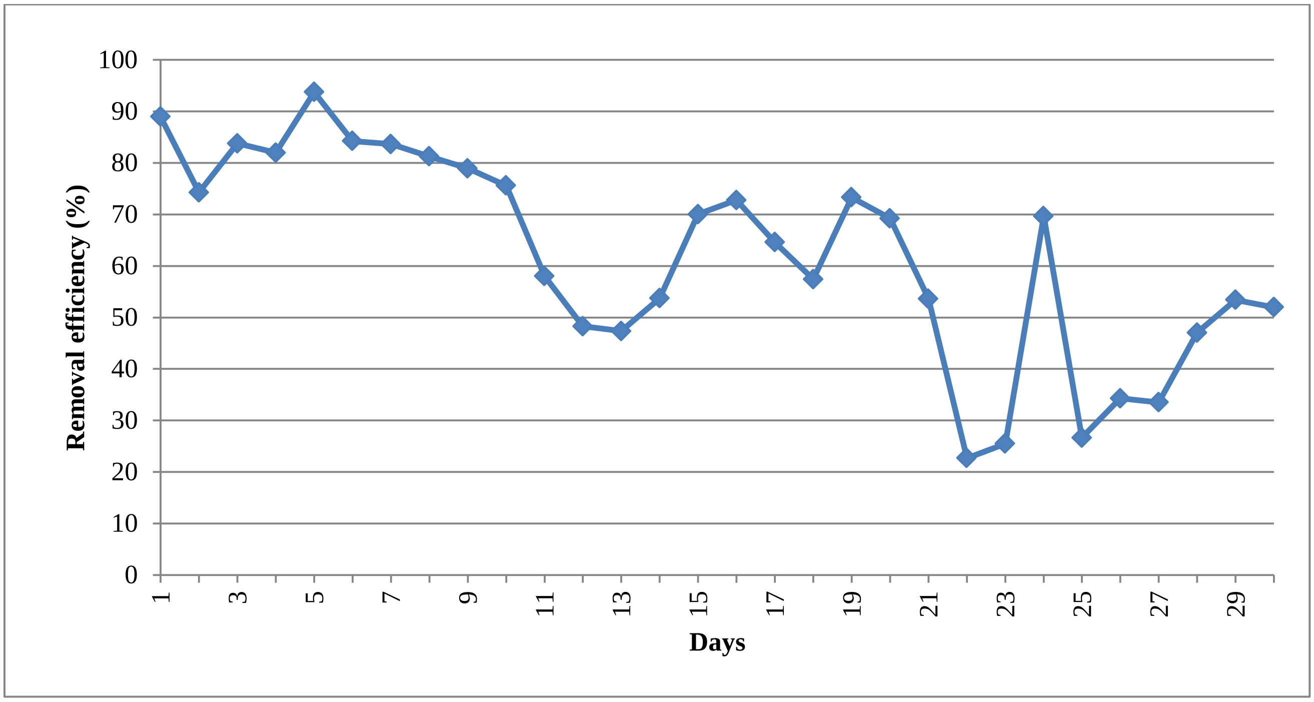

Figure. 4.3 Removal efficiency (%) of *Canna lily* (Total Phosphate)

In a study conducted by Govindrajan (2008)**,** removal of phosphate was found to be 16% in a constructed wetland which contains *Canna lily* as one of its plant species. The removal efficiency of TP ranged from 0-32% which varied over months. In July 2007, the removal efficiency was negative, which indicates that there might be internal loading of phosphorus in the system. Ni-Bin chang *et.al* (2010), conducted that *Canna lily* has a good potential to remove phosphorus in a wetland. In their study they have found phosphorus removal efficiency was 98.3%.

In the present study the average removal of phosphorus was found to be 61.13% which is much better than the study done by Govindrajan (2008). While the removal efficiency in the present study is not so good compared to the study done by Ni-Bin chang *et.al* (2010).

Removal of Available Phosphate (AP)

The influent concentration in the present study was varies between **0.97-4.64 mg/l** and the concentration in effluent varied from **0.28-2.80 mg/l** with an average value of **1.06 mg/l**. The major removal mechanism of Available Phosphorus (AP) was mainly absorption of phosphate which was available for the plant in the form of orthophosphate.

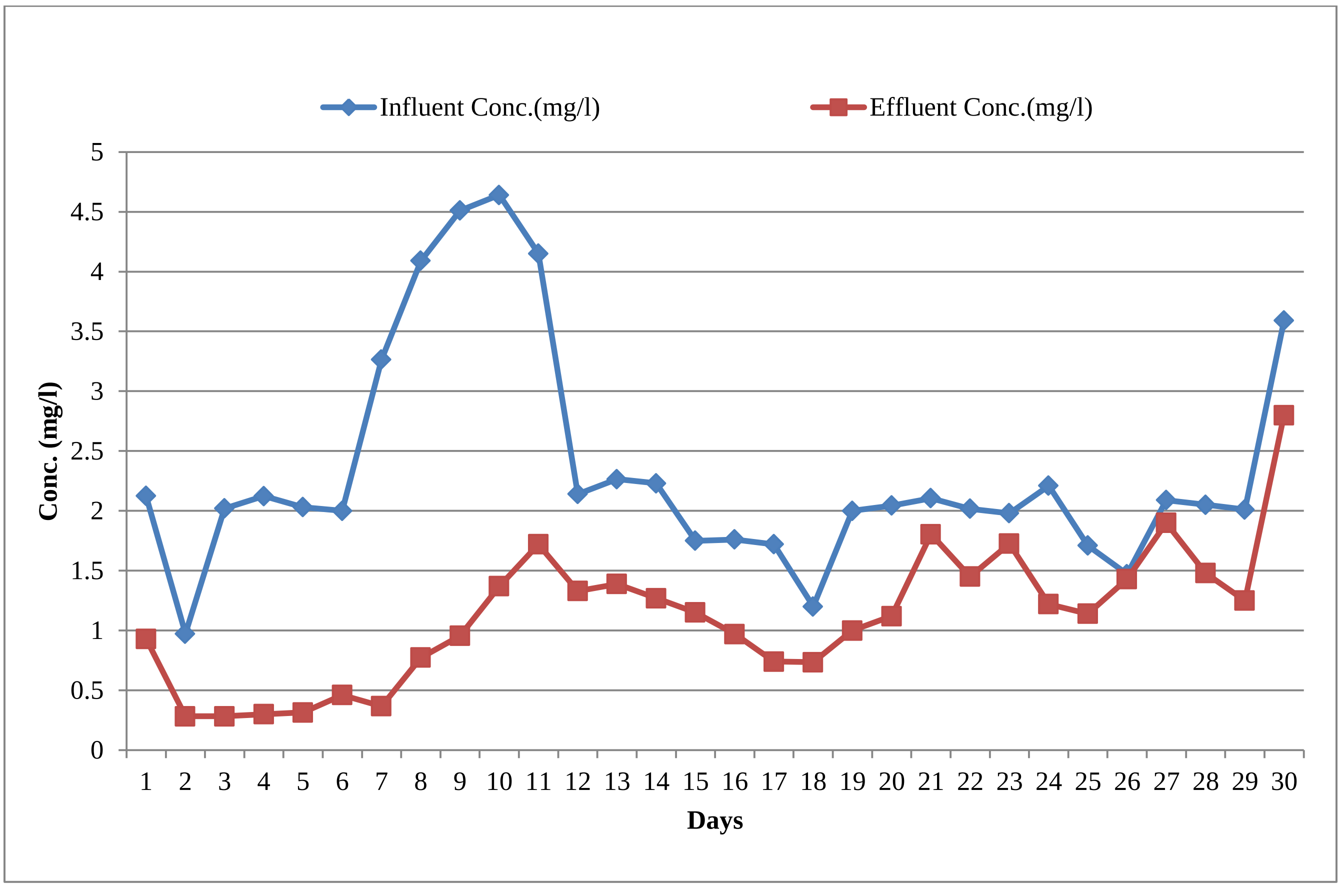

Figure. 4.4 Influent and Effluent concentration (mg/l) of Available Phosphate

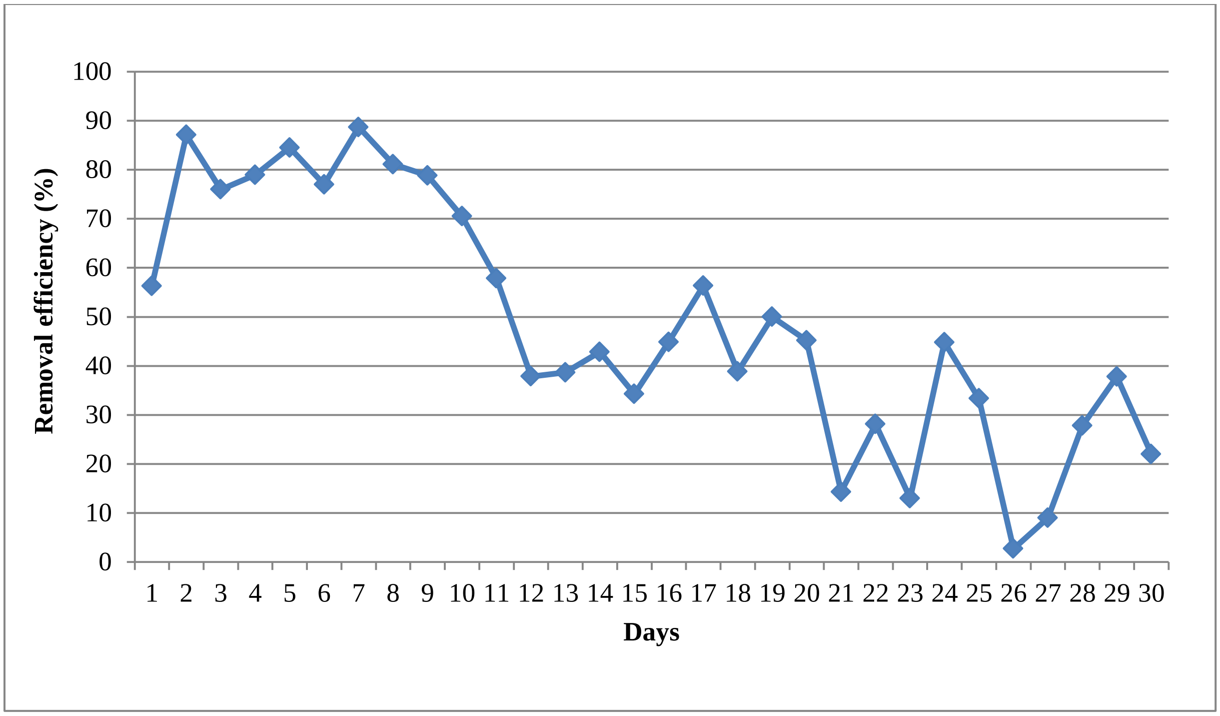

Figure. 4.5 Removal efficiency (%) of *Canna lily* (Available Phosphate)

In this study removal efficiency of AP varied over a wide range 2.7%-88.7%. The lowest efficiency was found on Day-26 (14th May`13) that is 2.7% with a sharp decrease from 44.81% (Day-24) and again increase to 37.2% (Day-29)**.** This wide variation might be correlated to the mechanical damage of the plants in the wetland system.

Table 4.2 Percent Removal of TP & AP by *Canna Lily*

Date	Retention Period (hours)	Total Phosphate (TP)			Available Phosphate (AP)		
		Influent Conc. (mg/l)	Effluent Conc. (mg/l)	Removal Efficiency (%)	Influent Conc. (mg/l)	Effluent Conc. (mg/l)	Removal Efficiency (%)
2nd April`13	24	8.43	0.92	88.97	2.12	0.92	56.25
4th April`13	48	5.28	2.19	74.25	0.97	0.28	87.10
5th April`13	24	4.67	0.66	83.78	2.02	0.28	75.99
8th April`13	72	4.56	0.82	81.97	2.12	0.30	78.96
9th April`13	24	6.64	0.41	93.75	2.03	0.31	84.50
10th April`13	24	6.33	0.99	84.23	2.00	0.46	77.00
11th April`13	24	6.17	1.01	83.66	3.26	0.36	88.70
12th April`13	24	6.66	1.24	81.27	4.09	0.77	81.10
15th April`13	72	6.30	1.32	78.94	4.51	0.95	78.82
16th April`13	24	7.50	1.83	75.60	4.64	1.37	70.50
17th April`13	24	6.00	2.52	58.00	4.15	1.72	57.83
18th April`13	24	4.81	2.48	48.27	2.14	1.33	37.85
19th April`13	24	6.21	3.27	47.34	2.26	1.38	38.65
23rd April`13	96	4.31	1.99	53.71	2.23	1.27	42.85
24th April`13	24	7.07	2.12	70.05	1.75	1.15	34.26
25th April`13	24	5.84	1.59	72.77	1.76	0.97	44.88
26th April`13	24	7.40	2.62	64.59	1.72	0.74	56.32

27th April`13	**24**	**5.84**	**2.49**	**57.40**	**1.20**	**0.73**	**38.83**
1st May`13	**96**	**5.21**	**1.39**	**73.30**	**2.00**	**1.00**	**50.00**
2nd May`13	**24**	**6.89**	**2.12**	**69.23**	**2.04**	**1.12**	**45.20**
3rd May`13	**24**	**4.51**	**2.09**	**53.63**	**2.10**	**1.80**	**14.29**
6th May`13	**96**	**3.52**	**2.72**	**22.70**	**2.01**	**1.45**	**28.11**
9th May`13	**96**	**4.68**	**1.42**	**69.65**	**2.21**	**1.22**	**44.81**
10th May`13	**24**	**3.91**	**2.88**	**26.65**	**1.71**	**1.14**	**33.33**
14th May`13	**96**	**3.12**	**2.05**	**34.29**	**1.47**	**1.43**	**02.70**
15th May`13	**24**	**5.01**	**3.23**	**33.50**	**2.09**	**1.90**	**09.00**
16th May`13	**24**	**4.11**	**2.17**	**47.02**	**2.05**	**1.48**	**27.80**
17th May`13	**24**	**3.87**	**1.80**	**53.40**	**2.01**	**1.25**	**37.80**
18th May`13	**24**	**5.33**	**2.56**	**51.97**	**3.59**	**2.80**	**22.00**
Range		**8.43-3.12**	**3.27-0.42**	**93.75-22.70**	**4.64-0.97**	**2.80-0.28**	**88.70-2.70**
Mean ± SD		**5.12±1.35**	**1.77±0.83**	**61.13±11.2**	**2.275±0.85**	**1.06±0.62**	**48.20±14.1**

4.3 Analysis of Sediment Samples

Analysis of sediment is one of the major parameter of this present study. The amount of phosphorus present in sediment has been the subject of a number of studies because of its role in the state and development of lake ecosystems. In lakes where the external loading has been reduced, internal phosphorus loading may prevent improvements in lake water quality. At high internal loading, particularly in summer concentrations will rise, and phosphorus retention can be negative during most of the summer (Martin Søndergaard *et.al* 2003). Internal phosphorus loading originates from a pool accumulated in the sediment at high external loading, and significant amounts of phosphorus in lake sediments may be bound to redox-sensitive iron compounds. These forms are potentially mobile and may be released to the lake water.

This present study has also been considered various fraction of phosphorus present in the sediment. To differentiate the sediment phosphorus fractions: associated to Al, Fe and Mn oxides and hydroxides, associated to Ca, organic and residual were analyzed separately. Five of the fractions mentioned non-apatite inorganic phosphorus (NAIP), bound to Al, Fe and Mn ox hydrates; apatite phosphorus (AIP), bound to Ca; inorganic phosphorus (IP); organic phosphorus (OP) and total phosphorus (TP) are attempted. The samples were collected when experiment was not started and when experiment was over from a single station (i.e. wetland). The physical characteristics were expected to be same but there may be change in Total Phosphate content as well as various fractions present in the sediment sample.

Characteristics of Sediments

Table 4.3 shows the main characteristics of the sediment in the wetland system. The soil sample had a specific gravity (G) of 2.502 with a (silt + clay) content of 18% where as sand content was 82%. Fig. 4.5 shows the particle size distribution of the soil sample. The soil sample can be characterized as Silty Sand (SM). The void ratio (e) of the sample was 0.19 and the water content was 7.6%. The liquid limit, plastic limit and plasticity index were 24%, 20%, 4% respectively.

Table 4.3 Summary of Laboratory Test Results

Sample	Summary of Laboratory Test Results											SOIL TYPE	Liquid Limit (%)	Plastic Limit (%)	Plasticity Index	COHESION 'C' in t/m^4
	Sieve Size in mm								Sample Constituent							
	4.75	2.00	1.18	0.60	0.43	0.30	0.15	0.075	Gravel (%)	Sand (%)	Silt + Clay (%)					
	100	98	93	81	78	64	38	18	0	82	18	Silty Sand (SM)	24	20	4	0

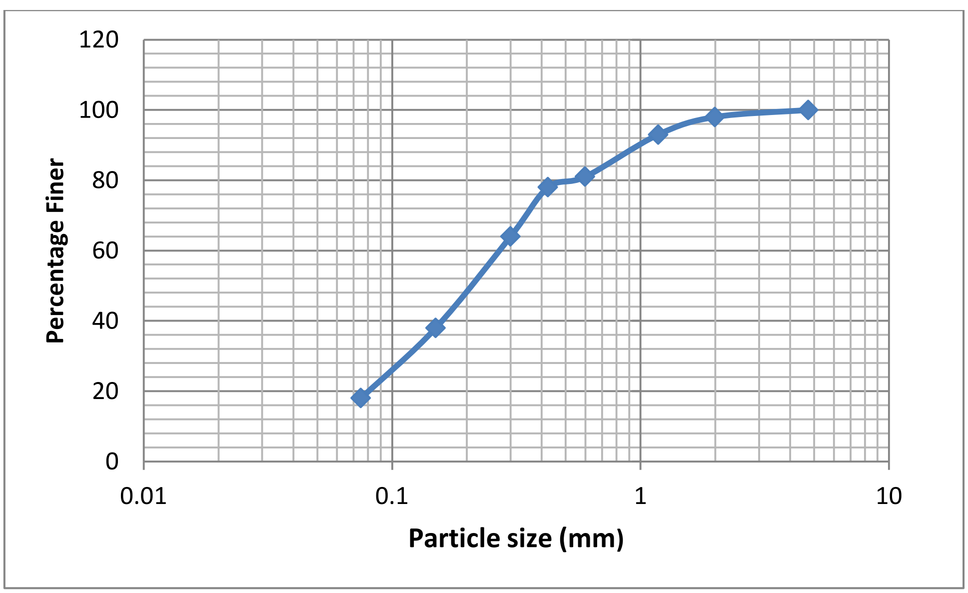

Figure. 4.6 Particle size Distribution of Sediments

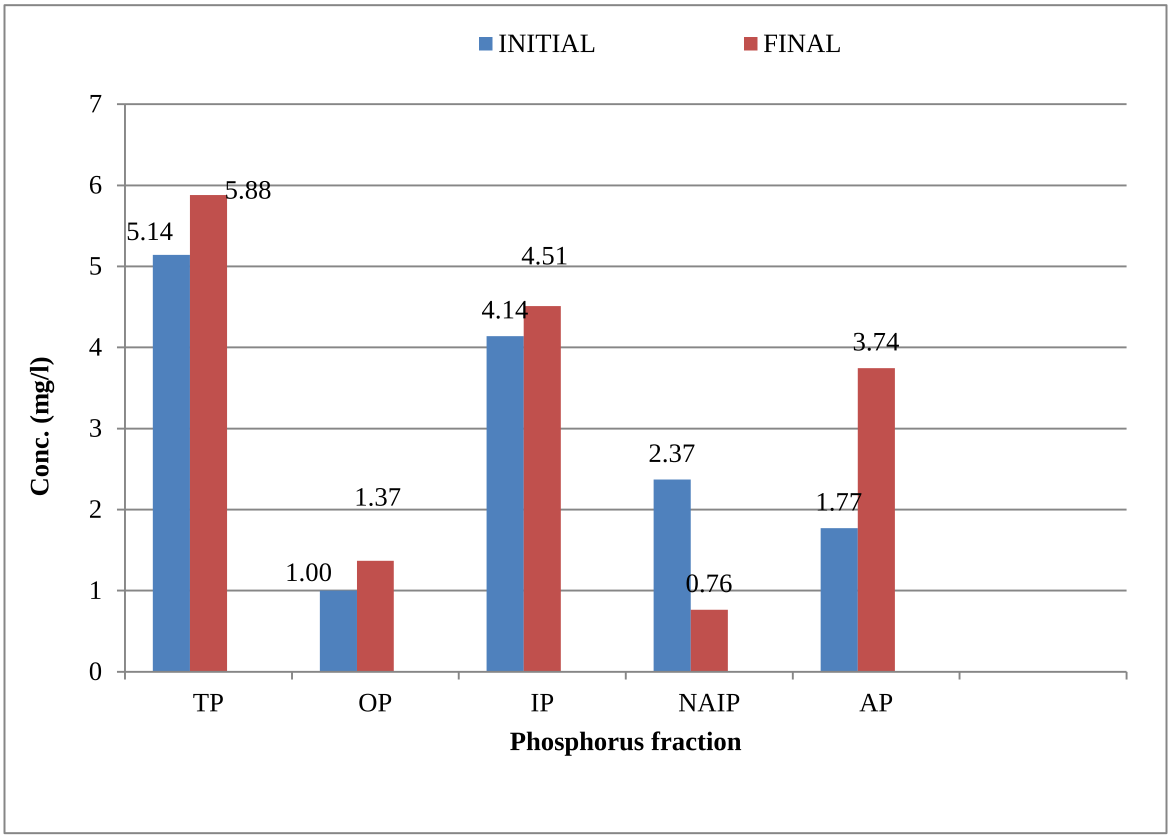

Figure.4.7 Comparison study between Initial and Final sediment samples

In this comparison study two sediment samples have been considered before and after the experimental period of 30 days. It is clear from Fig.4.7 that concentration of total phosphate (TP) will increase within experimental period. It will indicate that sediment may be taking part in phosphate removal. The other fraction of phosphate will also increase except NAIP. NAIP-is that fraction of phosphorus which is bound to Fe, Mn, Al, which may indicate release of phosphate from the sediment to the system.

In a study done by Martin Søndergaard (2003) on role of sediment in internal loading of phosphorus, where he described phosphorus which is accumulated in a pool sediments may be bound to redox-sensetive iron compounds. According to his study this form is potentially mobile and may be released to the pool water.

In another study done by Bin Zhang *et.al* (2011) the phosphorus sorption and release characteristics of the surface soil in the water-level-fluctuating zone were investigated. The results indicated the rank order of P-fraction was AIP> Organic P>NAIP. Similarly in this study the same order has been found for final sediment sample.

Table 4.4 Faction of Phosphate Present in Sediments before and after the Study

P-fraction	Initial sample (mg/l)	Final Sample (mg/l)
Total Phosphate (TP)	**5.14**	**5.88**
Organic Phosphate (OP)	**1.00**	**1.37**
Inorganic Phosphate (IP)	**4.14**	**4.51**
Non-Appetite Inorganic Phosphate (NAIP)	**2.37**	**0.76**
Appetite Phosphate (AIP)	**1.77**	**3.745**

The study indicated that the sediments play a significant role in removal of phosphate from waste water. The retention of phosphate is done by chemical binding with calcium (Ca) present in the sediment. The phosphate bound to Mn, Al, and Fe in sediments is related to natural redox reactions which play a role in it.

4.4 Analysis of Plant Tissue

Since phosphate removal efficiency will depend upon the plant (*Canna lily*) or more specifically said as phosphate uptake efficiency by the plant, it becomes important to know the accumulation of phosphorus in various parts of plant tissue. In this study two samples of plant Initial plant sample i.e. plant sample collected before the experiment and Final plant sample i.e. after the completion of the experiment were collected. The accumulation of phosphate in various parts of plant were determined to know which part of the plant will be more affected due uptake of phosphate from waste water. Fig. 4.8 will show the accumulation of phosphate in various part of initial sample and final sample respectively. In case of initial as well as final sample maximum phosphorus concentration found in "stem" and minimum concentration was found in "leaves". It may indicate the phosphate which is taken by the plant is used for their growth and metabolism. Fig. 4.8 a comparison between two samples. Phosphorus accumulation will increase in considerable amount in case of leaves and flowers.

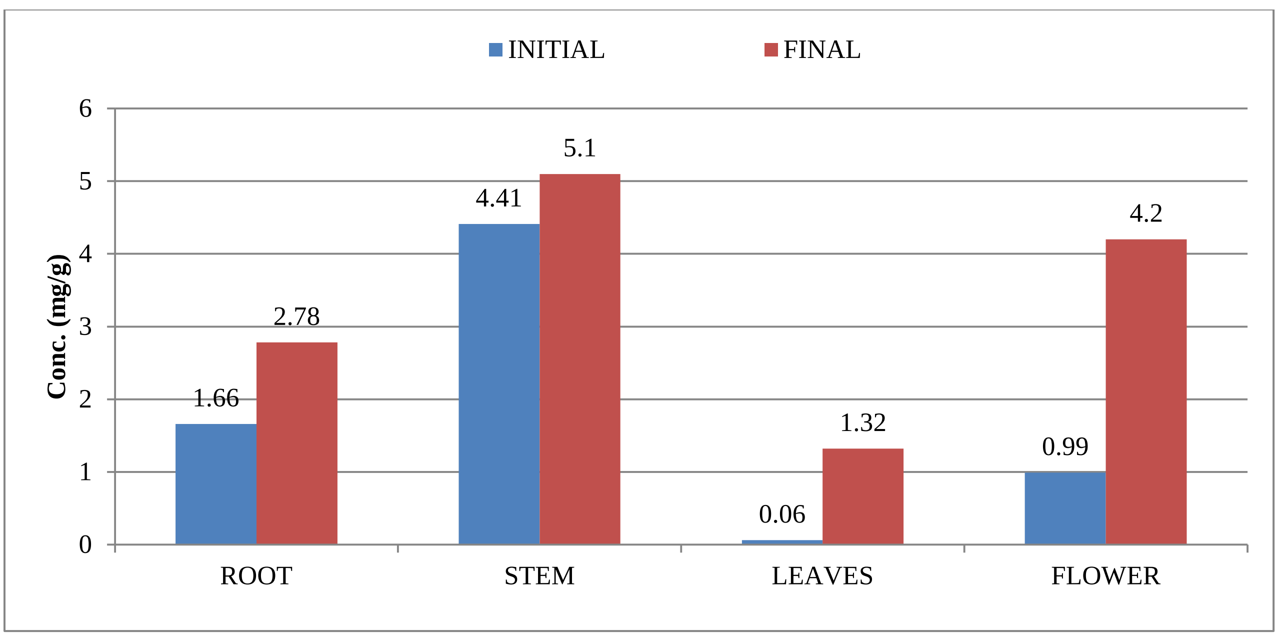

Figure. 4.8 Total Phosphate Concentration (mg/l) in Different Plant tissues

4.5 Effect of wastewater on *Canna Lily*

Since the nutrients like phosphate are taken by the plant for its growth and metabolism, it becomes important to monitor the growth of plant during the study period. In this present study the growth of plant was monitored in terms of root length, shoot length, shoot: root ratio and plant density. Table-4.5 will show these various parameters in detail, plant density had increased by 2.04 times where as shoot length and root length increased by 3.67 times and 3.69 times respectively.

Table 4.5 Characteristics of *Canna lily* before and after the experiment

PARAMETER	Day 1 (Initial)	Day 30 (Final)
Shoot length (cm)	**43.18**	**157.48**
Root length(cm)	**32.50**	**120.00**
Shoot: Root ratio	**1.33**	**1.32**
Plant Density (unit/m^2)	**110.50**	**225.56**

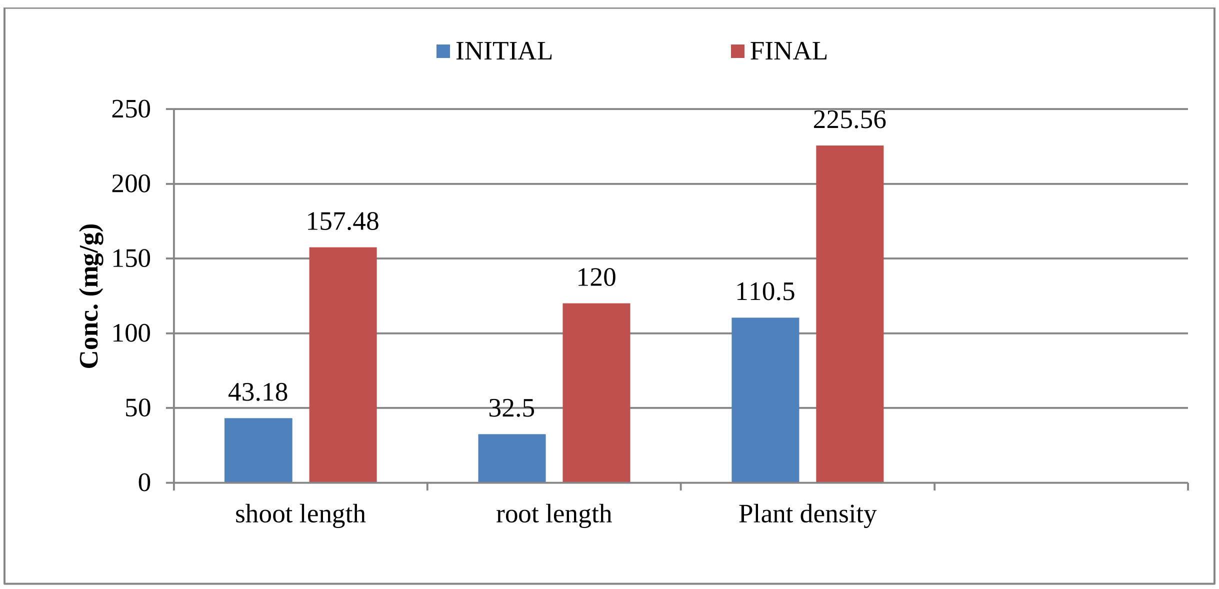

Figure. 4.9 Growth parameters of *Canna lily* before and after the experiment

Fig. 4.9 shows increment in the root length, shoot length, and plant density. This indicates that there will be no stress on plant by the waste water and their growth is normal. In this study the species of *canna lily* planted in the wetland had shown good growth and density of plant per unit area also increased. This indicate that waste water containing phosphate of a concentration ranges between 3ppm-8ppm does not hinder the growth of plant but promotes it. This indicate that phosphate present in waste water has a positive effect on the growth and reproduction of *Canna lily*. The removal of phosphate from wastewater might be because of interaction between plant, microbe sand wastewater. Plants have taken phosphate from waste water for their growth and metabolism. Microbes present in soil liberate various enzymes which convert various forms of phosphate into available from of phosphate. It will help in growth of plant as well as removal of phosphate from waste water.

4.6 Conclusion

Based on the results obtained in the present study following conclusions were made.

1) The plant *Canna lily* can survive under semi-arid conditions in a temperature range of 37˚ C-27˚ C. It can be used for phosphate removal from secondary- treated waste water under Indian conditions with an avg. efficiency ≈ 60%.

2) There is an active mobilization of phosphate in waste water-sediment-plant system. Most of the phosphate in sediment remains as in organic phosphate. Available phosphate (AP) in waste water gets transform to Appetite phosphate (bound to Ca), the Non-Appetite Inorganic Phosphate (NAIP) on the other hand, is generally release to waste water as a result of different redox reactions involved in natural aquatic chemistry.

3) The Total Phosphate concentration (mg/g) in different tissues of plant increased and revealed that it plays an important role in growth and metabolisms of plants. Most of the phosphate accumulates in leaves and flower and remaining in root and stem.

4) The waste water with phosphate concentration in the range of 3-8 mg/l had no stress over the plant. There was a significant increase in root length (3.69 times), soot length (3.67 times), and plant density (2.04 times) during study period.

5) *Canna lily* is a promising tool in phosphate removal from waste water with an average efficiency of 60% without any stress of influent concentration (phosphate) and temperature fluctuations. The plant can be used in stabilization of waste water under remain conditions.

2. Summary

A wetland is an area of land whose soil is saturated with moisture either permanently or seasonally, such that it takes on the characteristics of a distinct ecosystem. The capacity for wastewater purification by both natural and artificial wetlands is well documented. Wetlands remove pollutants through a complex variety of biological, physical, and chemical processes. There are many advantages of using constructed wetland over conventional system. The major problem in conventional system is the disposal of secondary pollutants (like Sludge) where as in wetland there is no such problem**.** As the application of constructed wetland (CW) as an advanced treatment, the usual aim is to remove nutrients (phosphorus & nitrogen compounds) which are detrimental to the balance of more sensitive receiving media where it may result in euthrophication. Wetlands are cheaper to construct than any other conventional treatment option. Operation and maintenance cost (energy and supplies) are lower in the case of CW since it is a natural process and it does not require continuous monitoring. Wetland treatment system use different water tolerant plant species. The basic types of constructed wetland treatment systems according to flow type include surface flow (SF) wetlands and subsurface flow (SSF) wetlands. There are three broad categories of wetland vegetation; 1) Emergent Aquatic Macrophytes (like- Typha,Canna lily), 2) Floating-leaved aquatic macrophytes (i.e. Water hyacinth, Duckweed), 3) Submerged aquatic macrophytes. Most of the research on constructed wetlands has been conducted in European countries. As a result, the plants most studied for treatment purposes are those which can survive in cold conditions. However, there is little information about the efficacy of ornamental plants for use in treatment wetlands. The fact that these ornamental plants cannot survive the cold winters in northern countries may be one reason why they have had limited use in constructed wetlands. The use of ornamental plants in treatment wetlands has been explored in this study. Keeping this fact in mind the present study will mainly focused on phosphorus removal efficiency of *Canna lily* which has shown a high promise towards the removal of organic matter and nutrients. Another important objective of this study is to get an idea of various fractions of phosphate in wastewater, plant, and sediments.

In this present study *Canna lily* is planted in a bench-scale wetland system with dimension 110cm×80cm×45cm and a constant flow rate maintaining a Sub-Surface flow condition with a hydraulic retention time of 1 day. There are three experiments has been done over this wetland system. In 1st experiment waste water analysis has been done which shown an average removal efficiency of 60% without any stress on the plant of influent concentration (3-8 mg/l) and a temperature fluctuation (27-37˚C). 2nd experiment carried over sediments collected from the wetland. Various fractions of phosphate present in sediment and mobilization of phosphate in sediment to water has been studied is this experiment. The results indicated the rank order of P-fraction was Appetite Phosphate (bound to Ca)> Organic P>Non-Appetite Inorganic Phosphate (bound to Fe, Mn, Al). Available Phosphate (AP) in waste water has a tendency to transform to Appetite Inorganic Phosphate (AIP) where as NAIP bound to redox-sensetive iron compounds are potentially mobile and may be released to water. In 3rd experiment analysis of plant tissue has been done to know the accumulation of Total Phosphate in various part of plant tissue to get an idea overall effect on the plant (*Canna lily*) during study period. The phosphate concentration (mg/g) in roots, stem, leaves, and flower has been studied which shows maximum accumulation of phosphate in leaves followed by flowers, stem, roots which indicate phosphate will taking part in metabolism and growth of the plant. The increased root length, soot length, and plant density will also indicate there was no adverse effect on the plant due to application of waste water. The conclusion may be drawn from the present study that *Canna lily* is an advanced and promising tool in phosphate removal from waste water with an average efficiency 60% without any stress.

Though the plant *Canna lily* has good potential towards the removal of phosphate from waste water under bench-scale study, with a short period of time (May`13-April`13) but the efficiency towards removal may vary/decrease under field conditions because of the varied nature of chemical species, their interaction, temperature variation and variation in humidity over the whole year. Presence of heavy metal and pesticides in waste water can significantly reduce the plant growth and may result in lower removal. Therefore, the study may be extended to study the effect as well as removal efficiency for actual field condition over a huge fluctuation of temperature and humidity.

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