INTRODUCTION

Man is an integral part of the environment, and there is no denying to the fact that his activities are hampering the environment. The major cause of concern due to these activities is related to pollution. Due to increasing population densities and impact from human activities, pollution remains an essential issue for water quality protection and conservation of biodiversity in urban areas. The objective of the study is to provide a comprehensive solution for the improvement of water quality, wastewater purification, enhancement of biodiversity and providing a cost effective method for wastewater disposal and treatment.

As we know water quality is an essential parameter, and usually the level of pollution in an effluent exceeds the standards. Many a times, however, a region does not have a purification system or lacks the capacity or technology to treat all the effluent due to the high cost of treatment system. This sometimes results in municipal and industrial sewage to be directly discharged into aquatic environment such as rivers, streams, lakes etc. with no treatment.

The conventional wastewater treatment system usually involves various physico-chemical treatment methods which involves high operating cost, greater maintenance and requires skilled labour for proper operation and functioning. Such system hampers the surrounding environment during the treatment process and land allocation to such units affects the bio-diversity of the area, hampering the aesthetics. An alternative to such conventional system is Constructed Wetlands (CW) which not only is cost efficient and easier to operate without skilled labour and maintenance, but also improves the aesthetics and biodiversity of the region concerned.

Wetlands are known as the most productive ecosystem in the world. Countless species of birds, mammals, reptiles, amphibian, fish and invertebrates depend on water and vegetation in wetland habitat. Wetlands also directly and indirectly support people by providing ecosystem services such as flood abatement, food, a clean water supply, aesthetic beauty, recreational and educational benefits and a source of carbon sink. This ability to simultaneously achieve water quality improvement and enhance biodiversity has generated an increasing interest in constructing and restoring wetlands for wastewater treatment.

The capacity for wastewater purification, by both natural and artificial wetlands, is well documented (Stephenson *et al*. 1983; De Busk, 1999; Vymazal, 2006) Wetlands remove aquatic pollutants through a complex variety of biological, physical and chemical processes. Although the higher aquatic plants are the most obvious biological component of the wetland ecosystem, recent reports in the literature point to the fact that uptake of pollutants by wetland vegetation cannot by itself account for the high pollutant removal efficiencies often observed at the high loading rates characteristic of many treatment situations (Stephenson et al., 1980). Rather, the major mechanisms for pollutant removal in these wetland systems include bacterial transformations and physico-chemical processing including adsorption, precipitation and sedimentation (Chan et al., 1982). This is not to say however, that the higher plants do not play an important role in wastewater purification. In particular, the plant rhizome provides surfaces for bacterial growth as well as for filtration of solids.

Nitrogen and phosphorous are identified as pollutants regulating the health of wetland vegetation. 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

There have been limited studies on removal of phosphorous from wetland using emergent plants that can be used as fodder. The plants studied till date find final disposal in composting, handicraft or are burnt. Plants such as *Brachiaria mutica* can find an application in removal of nutrients and later serve as cattle fodder. Keeping in view the above said observation, it was decided to undertake a research on wastewater treatment using a very commonly found wetland species of grass, namely *Brachiaria mutica* commonly known as para grass.

Brachiaria mutica belongs to family of Poacea (alt. gramineae). It is a common variety of C4 plant and is also known as para grass, buffalo grass, Dutch grass, giant grass, Mauritius signal grass etc. to name a few. They are widely available in Africa, Australia and India. These plants are well adapted to a wide range of soil of moderate to good fertility and are especially suited to poorly drained lands(such as in swampy or wetland areas). These species are highly tolerant to moderate salinity, low pH upto 4.5 and high levels of trace elements normally produced under water-logged conditions.

Brachiaria mutica plant was chosen because even though many wetland species such as canna lily, duckweed, cattails etc. have been researched upon, this particular species of invasive grass has been overlooked, even though this plant has higher adaptability, better suitability to wetland environment and has higher productivity, but has not been researched upon. The plant is easily available in India and can be studied.

Another aspect of this study was to get an idea about the various fraction of phosphorous present in wastewater and treated water.

Therefore, the present study is undertaken with the following objectives

- To study the phosphate removal efficiency by *Brachiaria mutica.*
- To study removal efficiency in different seasons.
- To study the various fractions of phosphate in wastewater.
- To suggest the efficiency of *Brachiaria mutica* for phosphate and nitrate removal from wastewater.

REVIEW OF LITERATURE

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. It represents the transition zone between terrestrial and aquatic environments. The average water depth which typically separates wetlands from adjacent aquatic system is in the range of 1 to 2m.

In wetland, micro organisms convert the biodegradable complex substance into simpler forms. This is termed as bioremediation. When plants convert the complex substance into simpler form by enzymatic action or any other processes like oxidation or reduction it is termed as phytoremediation, which is a part of bioremediation.

2.1 Types of Wetland

Ecological engineering, including the employment of constructed wetlands and the culture of aquatic macrophytes, for the purpose of pollution abatement has received growing acceptance (Reddind *et al.,* 1997). Wetlands are cheaper to construct than any other conventional treatment option. They can be broadly classified as natural wetlands and constructed wetlands (CW).

Natural wetlands include swamps, marshes and bogs among others. Natural wetlands are considered the most biologically diverse of all ecosystems. Plant life found in natural wetlands includes mangroves, water lilies, cattails, sedges, tamarack, blue spruce, cypress, gum and many others. Animal life includes many different amphibians, reptiles, birds, insects and mammals.

In constructed wetlands, operation and maintenance cost (energy and supplies) are lower than the conventional treatment process, 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 used for energy production in separate industry. There are no foul odours or harmful by-products developed from this system. The plants also do some environmental service by fixation of carbon dioxide and production of oxygen.

There are two major types of constructed wetlands. The first is called a Free Water Surface Wetland (FWS) and the second type of CW is a Vegetated Submerged Bed Wetland (VSB).

2.1.1 Free Water Surface Wetland

An FWS wetland encompasses shallow water flowing over plant media and water depths that vary through the wetland. Typically these wetlands resemble natural wetlands and include mineral or organic soil underneath vegetation. Vegetation includes reeds and cattails, but can also include floating plants which are also known as macrophytes.

2.1.2 Vegetated Submerged Bed Wetland

A VSB contains coarse substrate media such as gravel through which the water travels. The top of the water level is below the surface of the media and plant roots are allowed to grow in the coarse media. These wetlands remove contaminants by different means but the basic processes and mechanisms are the same for both.

Wetlands can also be classification based on flow of wastewater. Accordingly, they are classified as Surface Flow (SF) and Sub-Surface Flow (SSF)

2.1.3 Surface Flow

In surface-flow wetlands, wastewater flows above the soil in a planted marsh or swamp, and thus can be supported by a wider variety of soil types including bay mud and other silty clays.

2.1.4 Sub Surface Flow

Subsurface-flow wetlands can be further classified as horizontal flow and vertical flow constructed wetlands. Subsurface-flow wetlands, effluent (household wastewater, agricultural, paper mill wastewateror mining runoff, tannery or meat processing wastes, or storm drains, or other water to be cleansed) flow through a gravel (generally limestone or volcanic rock lava stone) or sand medium on which plants are rooted. In subsurface-flow systems, the effluent may move either horizontally, parallel to the surface, or vertically, from the planted layer down through the substrate and out. Subsurface horizontal-flow wetlands are less hospitable to mosquitoes, (as there is no water exposed to the surface) whose populations can be a problem in surface-flow constructed wetlands. Subsurface-flow systems have the advantage of requiring less land area for water treatment, but are not generally as suitable for wildlife habitat as are surface-flow constructed wetlands.

2.2 Wetland Vegetation

Wetlands are dominated by plant species that are adapted to growing in seasonally or continuously flooded soil with resulting anaerobic or low oxygen conditions. There are three broad categories of wetland vegetation.

2.2.1 Emergent Aquatic Macrophytes

These are plants that include species which are rooted in the substrate. They grow within a water table range of 50 cm below the soil surface to 150 cm or more, like-Typha, Canna lily

2.2.2 Floating Aquatic Macrophytes

These includes both species that live either completely submerged or floating or have some small portion of the plant emerging from the water and species which are free floating on the water surface e.g. Water hyacinth, Duckweed.

2.2.3 Submerged Aquatic Macrophytes

They have their photosynthetic tissue entirely submerged but usually the flower is exposed to the atmosphere.

2.3 Removal of Pollutants

Wetland treatment process is a combination of all unit process plus other physico-chemical processes, sedimentation, biological oxidation, nutrient incorporation, adsorption and inpercipitation (Gearheart, 1992)

Wetlands remove pollutants through a complex variety of biological, physical, and chemical processes aided with long detention time. Owing to extensive surface areas, wetland performance is affected by rainfall, evapotranspiration and temperature. Because these factors change over time, wetlands systems do not operate as a steady-state process. However 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.

Pollutant removal in wetland is achieved by physical, chemical and biological processes.

Physical processes are often used in primary treatment of traditional wastewater treatment system. The processes are no different than in wetlands. Water that flow through wetlands moves rather slowly due to resistance from plant matter. A study on wetlands by DeBusk (1999) observed that plants in wetlands help trap sediments. It also lowers the velocity of flow, which allows particles to settle out.

Chemical process includes sorption, photo oxidation and volatilization. Sorption is the most important chemical process and involves the moving of charges from aqueous phase to solid phases. Sorption includes adsorption and precipitation. Adsorption refers to transfer of ions to soil particles and precipitation involves converting metals to insoluble forms (Morton, 2005). Photo oxidation utilizes the power of sunlight to breakdown and oxidise compounds. Volatilisation breaks down compound and expels it into air as gaseous state(Gonzales; 2005).

Biological process is one of the most important mechanisms for removal of pollutants in wetlands (Interstate, 2003). It was noted that biological removal included a complex mechanism of phytodegradation, rhizodegradation, phytovolatilization.

In a study by Mitchell (1966), it was observed that there are six major biological reactions involved in performance of constructed wetlands including photosynthesis, respiration, fermentation, nitrification, de-nitrification and microbial phosphorous removal.

These physical chemical and biological methods and processes hence play a vital role in wastewater treatment. Various studies have been undertaken in the past regarding this also. Macrophyte-based wastewater treatment system were found to have several potential advantages compared to conventional treatment system (Brix and Schierup, 1989).

Plant species such as duckweed have been found to achieve high level of nutrient removal (Sutton and Ornes, 1975; Reddy and De Busk,1985; Tripathi *et al.*, 1991; Alaerts *et al.,* 1996) whilst low fibre and high protein content (Landolt, 1986; Hammouda *et al.*, 1995) make it a valuable fodder (Cully and Epps, 1973; Skillcorn *et al.,* 1993; Haustein *et al.,* 1994; Lang *et al.,* 1995). Duckweed has been found to suppress algal growth (Hammouda *et al.,* 1995) and have been studied for dairy waste lagoons (Cully *et al.,* 1981; Whitehead *et al.,*1987), raw and diluted domestic sewage, secondary effluent and fish culture systems. Several full scale systems are infact in operation in Taiwan, Bangladesh, China, Belgium and the U.S.A.

Several other wetland species have been studied upon for Constructed Wetland (CW) system. A study by Debing *et al.,* 2008 used *Typha-Phragmites-Scirpus* vegetation (with *T. Angustata, P. Communis, S. Validus* as major species), Typha main with T. Augustata as the major species and Typha monoculture vegetation as three design treatment plant in a pilot-scale gravel based subsurface wetlands to treat artificial sewage. The typha vegetation showed high COD, TN, TP removal loads in wetlands.

In another study by Cristina *et al.,* five plots were vegetated with *Canna Indica, Typha Latifolia, Phragmites australis, Stenotaphrum secundatum* and *Iris pseudacorus*. The treatment performance of the systems under two different hydraulic loading rates, 3 and 6cm/d was assessed. COD was observed to be reduced by 41-73% for an inlet organic loading varying between 332 and 1602kg/ha/d and BOD_5 was reduced by 41-58% for an inlet organic loading varying between 218 and 780kg/ha/d.

In another study by Sim *et al.*, five native species, *Typha angustifolia, Scripus mucronatus, Lepironia articulate, Eleocharis dulcis* and *Phragmites karka* were investigated for their nutrient removal efficiencies in shallow pond system in Buloh Wetland Reserve with water depth 0.1-0.30 m of atotal $160m^2$ in area size. The project aimed to investigate nutrient removal efficiencies, nutrient storage in plant biomass and plant growth. Pond water quality before and after planting in fully vegetated ponds improved significantly at 24.4% TP and 64.4% TN reduction. Nutrient concentration in leaves and stem samples of the five species were within the range of 1% to 3% of dry weight for nitrogen and 0.1%-0.3% of dry weight of phosphorous.

Average daily mass removal rates ranged from 3.5-9.6kg/ha/d to 13.5-33.5kg/a/day. This field trial showed that the four species, Lepironia articulate. Eleocharis dulcis, Typha augustafolia and scirpus mucronatus are suitable species in surface flow wetland or shallow pond system for nutrient removal in tropical environment.

2.3.1 Mechanisms of Suspended Solids Removal

The major processes involved for the removal of settleable suspended solids are sedimentation and filtration. Non settling/colloidal solids are removed partially by bacterial growth which results in the settling of some colloidal solids and the microbial decay of other, and collisions (inertial and Brownian) with the adsorption to the other solids (plant, pond bottom, suspended solids etc.) (Stowell *et al.,* 1981)

The two types of wastewater wetlands (FWS and VSB) offer different approaches at which suspended solids are removed from the system. The FWS wetland removes suspended solids primarily by flocculation/sedimentation and filtration/interception. Settling by gravity can be divided into discrete settling and flocculent settling. Both of these processes are influenced by particle size, shape, specific gravity, and properties of the fluid medium. Discrete settling is when a particle settles independently on its own with no contact from other particles. The settling velocities of these particles can be reasonably determined from Newton's Law and Stokes' Law. Flocculent settling cannot be as easily determined as discrete settling and must be found experimentally. Flocculent settling involves the interacting of particles changing size and characteristics. The formation of larger flocculants results from charge imbalances on the surface of the particles. If larger flocs are formed then the settling of the new particle will occur faster.

FWS wetlands can typically see hydraulic loads about .01 m/day to 0.5 m/day. The larger particles would be removed in the primary part of the wetland whereas the smaller particles may be flocculated by varying velocity gradients imposed by plant stems. Filtration does not typically play a large part in suspended solid removal of FWS wetlands since the plant stems of plants are too far apart. Interception and adhesion to plant surfaces play an important part in solid removal. The surfaces of plants in wetlands are coated with active layer of biofilm called periphyton which can absorb colloidal and soluble matter. These solids may then be metabolized and then converted to gases or biomass. The material then may renter the water column. There has been little research on this mechanism of removal in FWS wetlands. Typical total suspended solids concentration is around 3 mg/L. The mechanisms for FWS wetlands varies in regards to the mechanisms involved with VSB wetlands (EPA, 1999).

When dealing with removal of suspended solids in VSB wetlands, the main criteria that should be addressed are the hydraulic design and microbial characteristics of the substrate (Manios et al., 2003). Due to low velocity and large surface area of the media in VSB wetlands, they have proven effective in removing suspended solids. VSB wetlands offer gravity settling, straining, and adsorption onto gravel and plant media (EPA, 1999). It has been found that 60- 75% percent of solids removal in VSB wetland occur in the first one third of the wetland. One of the major concerns with VSB wetlands is the clogging of the filter media. As suspended solids pass through the soil media, it can clog pores and reduce the hydraulic conductivity of the media producing head losses at the entrance of the wetland. It is assumed that plant roots might help prevent clogging but no research has proven this.

Research of five different gravel based VSB wetlands being used for tertiary treatment in the United Kingdom have been analyzed over a two year period. Over the two years the wetlands removed an average 82% of the total suspended solids with an average effluent value less than 5 mg/L (Manios et al., 2003). VSB wastewater wetlands offer a great reduction in the amount of suspended solids but there are concerns with the clogging of the media. By using different types of media, clogging can be minimized and proper suspended solid removal can be achieved (Norton, 2010).

2.3.2 Mechanisms of Organic Matter Removal

Organic material is made up of about 50% carbon which microorganisms use an energy source. The vast arrays of microorganisms are adapted to aerobic surface waters or anaerobic soils. The aerobic microorganisms consume oxygen to breakdown organics which provides energy and biomass for the microorganism.

Anaerobic bacteria breakdown organic matter to produce methane. Biological oxygen demand is used to measure how much oxygen microorganisms are consuming to break down organics. It is important that there is enough oxygen in the water after the wetland so that plants and animals can survive. Wastewater wetlands are also capable of storing organic carbon in plant biomass thus making wastewater wetlands natural consumers of organic carbon (DeBusk, 1999b).

Organic removal is different for FWS and VSB wetlands but both share some of the same principles. FWS wetlands can remove organic matter by physical means and biological means. Physical removal is similar to suspended solid removal and the mechanisms are similar and it is not uncommon for effluent to have similar characteristics. The separation processes of organics include sorption and volatilization. The biofilms located on plant surfaces offer pathways for plants to break down organics. Although the amount volatile organic compounds entering wastewater wetlands is fairly low, the removal rate of VOCs are in the 80-96% range (Norton, 2010). The biological breakdown of organic matter is a very important one. Organisms will break down organic matter in order to produce new biomass, reproduce, and sustain life. Energy is a key element in any biological system and it can be in many forms. The main types of reactions with organic matter include aerobic, anoxic, and anaerobic (Daehler, 1997).

In an aerobic environment, oxygen is present and serves as the terminal electron acceptor. This is the most efficient conversion of starting material to end products. In an anoxic environment nitrates, sulphates, and carbonates serve as the terminal electron acceptor which are reduced to form oxides. Anoxic reactions are less efficient than aerobic reactions.

Anaerobic environments use the organics as the terminal electron acceptor and donor. The reactions the organisms use to break organics into energy are reactions that yield energy for the organism. These include oxidation and reduction reactions, hydrolysis, and photolysis. These reactions produce methane and are the least efficient of the three reactions. Benthos organisms are present on the bottom of the plant matter and in sediments. Periphytons are organisms that are attached to leaves and stems of rooted plants. Planktons are organisms that are dictated by currents. Neustons are organisms resting on the surface of the water. Nektons are organisms that have the capability to navigate the water on their own will. Bacteria, actinomycetes, and fungi play maybe the most important role in breaking down organic matter. Macrophytes are aquatic plants located on top of the surface of the water which play an important role in producing oxygen to the water (EPA, 1999). Figure below outlines the major organic matter transformations in a FWS wetland.

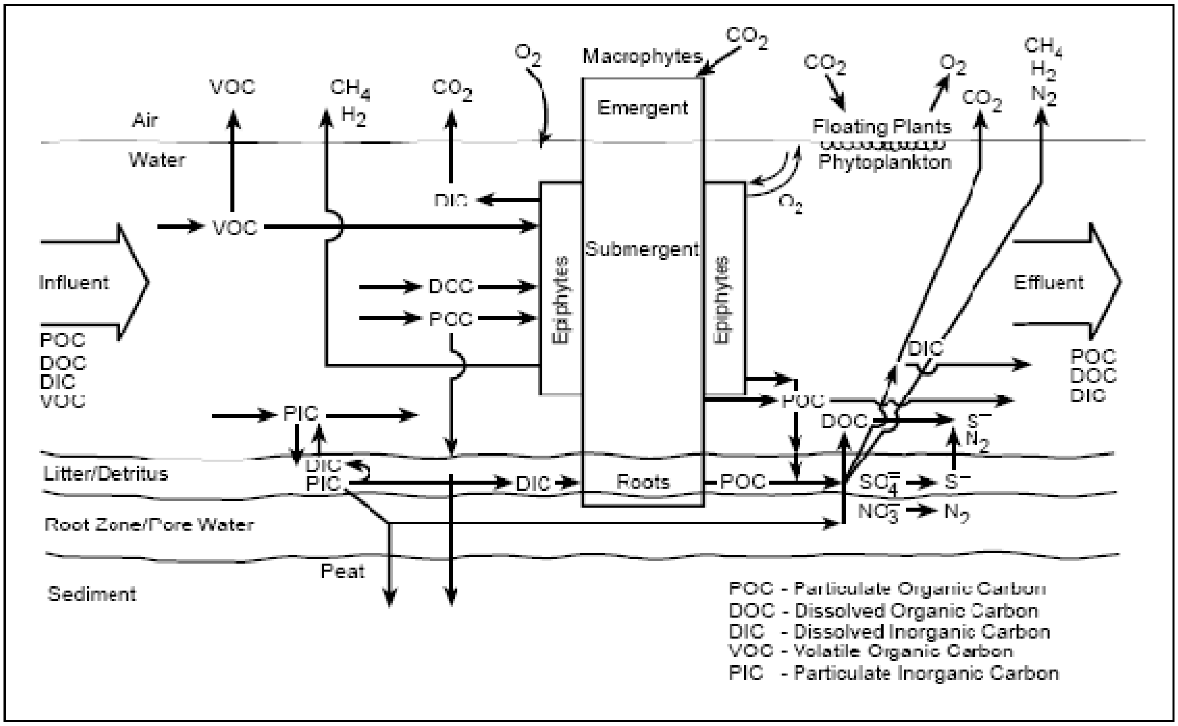

Figure 2.1: Major organic transformations in FWS wetland

The mechanism for organic removal in VSB wetlands is a little different than in FWS wetlands since VSB wetlands function as fixed film bioreactors. The particulate organic material entering a VSB wetland undergoes a similar mechanism as suspended solids. The particles undergo hydrolysis and produce soluble organic matter which enter the media and attach to biofilm media and then further decomposed.

The amount of decomposition of organic matter is rather low since average dissolved oxygen concentrations in a VSB wetland are less than 0.1 mg/L. The predominant biological mechanism for organic removal is done by aerobic/facultative means. VSB wetlands have strong reducing capabilities which make the predominant metabolic mechanism an aerobic manner. Anaerobic functions include methanogenisis, sulphate reduction, and gentrification which all produce gaseous products. These functions vary with temperature and it is possible that as temperatures increase greater amounts of gas can be released (EPA, 1999). The removal of organic matter drive dissolved oxygen concentrations but much attention has been aimed at nutrient removal due to eutrophication.

2.3.3 BOD, COD, TDS, TSS Removal Efficiency

The study constructed by Shah *et.al*. (2010), potential of Water Hyachinth (*Eichhorina crassipes*) in 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 non-vegetated beds, respectively. The mean effluent value of 22.3 mg/l for the reed bed was also significantly lower than the value for the non-vegetated bed. The cattail bed was again the poorest performer with regard to BOD removal, having a mean effluent level not significantly different from the non-vegetated 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 non-vegetated bed indicates that removal of suspended solids is almost due entirely due to physical processes (sedimentation and filtration) rather than biological processes associated with the microbial community or with the higher plants.

2.3.4 Mechanism of Nitrogen Removal

One of the important issues when treating wastewater is the removal of nitrogen. There have been many environmental and health problems associated with high amounts of nitrogen in water. High concentration of nitrates in drinking water can cause “blue baby” syndrome in infants. Ammonia that is not ionized can be toxic to marine organisms and aquatic life. High amounts of nitrogen also contribute to eutrophication in which nutrients promote excessive plant growth where plants deplete oxygen in the water. The need for proper nitrogen removal is very important.

Nitrogen exists in many forms such as inorganic and organic forms. Inorganic nitrogen includes nitrates, nitrites, and ammonium. In natural environments where oxygen is in surplus nitrogen usually exists as nitrates and nitrites. In environments that lack oxygen, nitrogen is available as ammonium which is the case in wetland soils. As nitrogen containing material settle in wetlands, the matter is either taken up by plants or broken down by microorganisms. Plants use nitrates and ammonium as nutrients which can be stored as organic nitrogen. When plants die the organic nitrogen present accumulates as peat as a long term storage mechanism. Microorganisms break down inorganic nitrogen mostly by de-nitrification which converts nitrate to nitrogen gas. If nitrogen is in the form of ammonium then this must be converted to nitrate by nitrification. Nitrate removal in wetlands is usually very high (Katsenovich *et. al.*, 2009b).

As per study by Vymazal in 2006, the removal of nitrogen involves a number of processes all which act on different types of wastewater wetlands. These processes include ammonia volatilization, ammonification, nitrification, nitrate-ammonification, denitrification, fixation, plant and microbial uptake, ammonia adsorption, organic nitrogen burial These are the major nitrogen mechanisms some of which occur in different types of wastewater wetlands (Vymazal, 2006).

As noted before the most important forms of organic nitrogen found in wetlands are ammonium, nitrate, and nitrite. These various forms of nitrogen are required for biological life to function in the wetland. The processes that transform various forms of nitrogen are all necessary for wetlands to function successfully.

Ammonia volatilization is the physicochemical process where ammonium is in equilibrium with gas and hydroxyl forms. Usually if the pH is lower than 8.0 and ammonia volatilization does not occur. If the pH reaches as high as 9.3 then ammonia and ammonium ions present exist in a one to one ratio. A larger pH can be observed when plants undergo photosynthesis during the day. According to the study by Reddy and Patrick (1984), the loss of NH_3 through volatilization from flooded soils and sediments are insignificant if the pH value is below 7.5 and very often losses are not serious if pH is below 8.0. At pH of 9.3 the ratio between ammonia and ammonium ion is 1:1 and losses via volatilization are significant.

According to study by Vymazal, the volatilisation rate is controlled by the NH_4^+ concentration in water, temperature, wind velocity, solar radiation, the nature and number of aquatic plants and the capacity of system to change the pH value in diurnal cycles.

Ammonification is the process where organic nitrogen is converted to ammonia. The process is biochemical which involves the release of energy which some microorganisms utilize for growth and new biomass. Up to 100% of organic nitrogen is converted to ammonia through a complex process involving the catabolism of amino acids. The process converts amino acids into ammonia by means of aerobically, anaerobically, and obligate anaerobically. The majority of ammonification is done by anaerobic and obligate anaerobic mineralization. According to the study by Reddy& Patrick, 1984, the rates of ammonification depend on temperature, pH, C/N ratio, available nutrients, and soil conditions. The optimal ranges include 40-60 degrees Celsius and pH between 6.5 and 8.5. the rate of aerobic ammonification doubles with a temperature increase of10˚C (Reddy *et. al.,*1979) This step is crucial before ammonium is then absorbed by plants, solubilized and returned to the water column, converted to gaseous ammonia, or aerobically nitrified by aerobic organisms (EPA, 1999).

Once organic nitrogen is in the form of ammonium, nitrification can take place where ammonium is biologically oxidized to nitrite and then finally to nitrate. Heterotrophic and autotrophic organisms utilize this process in the same manner. Usually nitrification has thought to be a chemoautotrophic process but recent research has shown that nitrification by heterotrophic means is also significant. Nitrifying bacteria utilize carbon dioxide as a carbon source and oxidize ammonia or nitrite to derive energy (Borin; 2012). Nitrification is carried out by two types of nitrifying organisms. The first step converts ammonium to nitrite and the second converts nitrite to nitrate. The first step is done firmly aerobically; the organisms depend on oxidizing the ammonia for cell growth and energy. Soil organisms include *Nitrosospira, Nitrosovibrio Nitrosolobus, Nitrosococcus*, and *Nitrosomonas*. The carbon source is mostly found from carbon dioxide but carbonate can be used as well. The second step converts nitrite to nitrate and is accomplished by facultative chemolitrotrophic bacteria which can utilize organics for cell growth an energy. The only organism found in soil of freshwater which oxidizes nitrites is *Nitrobacter*. Nitrification also is influenced by temperature, pH, alkalinity present, and dissolved oxygen. The ideal temperature is from 30 to 40 degrees Celsius. The pH values range from 6.6 to 8.8 and proper amounts of alkalinity and dissolved oxygen must be present. Nitrification consumes 4.3 mg of oxygen and 8.64 mg of alkalinity per mg of ammonia oxidized (Vymazal, 2006). Figure below outlines the major nitrogen transformations in a FWS wetland.

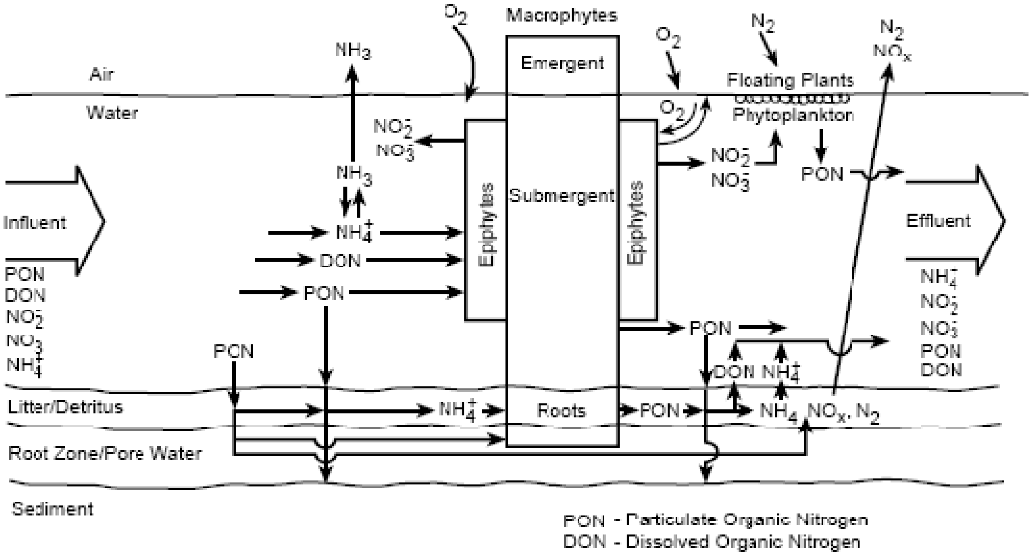

Figure 2.2: Nitrogen transformations in a FWS wetland.

Nitrate-Ammonification is the first anoxic process after oxygen is depleted in the system. This includes the reduction of nitrate to molecular nitrogen or ammonia (Norton, 2010). This is performed by two different nitrate reducing bacteria. Denitrifying bacteria produce dinitrogen oxide and nitrogen as major products. The second group produces ammonium as the major product from reduction of nitrate. *Bacillus, Micrococus*, and *Pseudomonas* are important denitrifying organisms in soils *and Pseudomonas, Aeromonas*, and *Virbio* are important in aquatic environments. In the presence of oxygen the organisms break down organics into carbon dioxide and water. The electron transport system enables the organisms to denitrify in anaerobic conditions.

Denitrification is a process that is similar to the aerobic process but takes place under anoxic conditions (Vymazal, 2006). Denitrification consumes 2.86 g of oxygen per gram of nitrate reduced as organic carbon is consumed. Alkalinity is replenished back into the system as about 3 grams of alkalinity is produced per gram of nitrate reduced (EPA, 1999). There are several factors which influence whether nitrification will take place. These include but are not limited to presence of oxygen, temperature, pH, and presence of denitrifiers. Under low oxygen levels the production of nitrite from ammonia is favored over nitrate formation from ammonia. This nitrite can still be denitrified to nitrogen gas without being converted to nitrate. A process that goes in the reverse way is called fixation.

Fixation is the process of converting nitrogen gas to organic nitrogen. The reaction can be done aerobically or anaerobically by certain types of bacteria and blue-green algae (EPA, 1999). There are a variety of wetland plants that can fix nitrogen but the process requires a large amount of cellular energy which is not necessary in a nitrogen rich environment (Vymazal, 2006). The process occurs on open water, in sediment, and on the leaves of plants. In natural wetlands fixation plays a more important role but not as important in constructed wastewater wetlands. This is because of the nitrogen rich environment that was created. An important part of nitrogen transfer in wetlands is plant uptake and assimilation. This refers to biological processes that convert inorganic nitrogen to organic nitrogen. The organic nitrogen is then used for energy and cell growth. The forms of nitrogen that are assimilated are ammonia and nitrate.

FWS wetlands have different ways of removing nitrogen than VSB wetlands since the media in which the water flows is different. Volatilization can be a large reducer of nitrogen in FWS wetlands if the pH of the systems increases. Ammonification does not decrease nitrogen but it gets organic nitrogen in the state of ammonia so that it can be removed by other processes. Ammonification is present in all types of wastewater wetlands. Nitrification also does not remove nitrogen but coupled with denitrification, the two are the major pathway for nitrogen removal. This process occurs in all types of wetlands but has been found to be the limiting step in nitrogen removal. This is because the vast amounts of nitrogen entering the wetlands are in the form of ammonia. Denitrification is the primary mechanism of removing nitrogen in wastewater wetlands. Usually the nitrate concentration of domestic wastewater is low, thus nitrification is crucial for proper denitrification. The amount of dissolved oxygen is also important when dealing with denitrification. Plant uptake is the primary mechanism for reducing nitrogen in FWS wetlands. The removal efficiency of all wastewater wetlands vary between 40 and 50%. This depends on whether the wetland is being used for primary treatment or secondary treatment. It has been found that wastewater wetlands may be unable to meet primary nitrogen requirements and that the system may better suited by coupling with a traditional wastewater treatment plant.

2.3.5 Mechanism of Phosphorus Removal

Another important nutrient that causes eutrophication in water is phosphorus. Removal of phosphorus tends not to be as high as nitrogen removal in wastewater wetlands. This is because wetlands do not provide the direct metabolic pathway to remove phosphorus. Wetlands use physical, chemical, and biological means to reduce phosphorus (DeBusk, 1999b). Phosphorus exists as phosphates as inorganic and organic forms. Biological oxidation results in conversion of most of the phosphorous to the orthophosphate form (Cooper *et al.,* 1996). The predominant form is in the form of orthophosphate which can be used by algae and macrophytes. Inorganic phosphorus can also be found as polyphosphates. Organic forms include phospholipids, nucleic acids, nucleoproteins, and phosphorylated sugars. These forms are primarily known as easily decomposable phosphorus and there other forms called slowly decomposable organic phosphorus which contains phytin (Vymazal, 2006). The major phosphorus transformations in wastewater wetlands are done by physical/chemical means and biological means.

Figure below outlines the major phosphorus transformations in a FWS wetland.

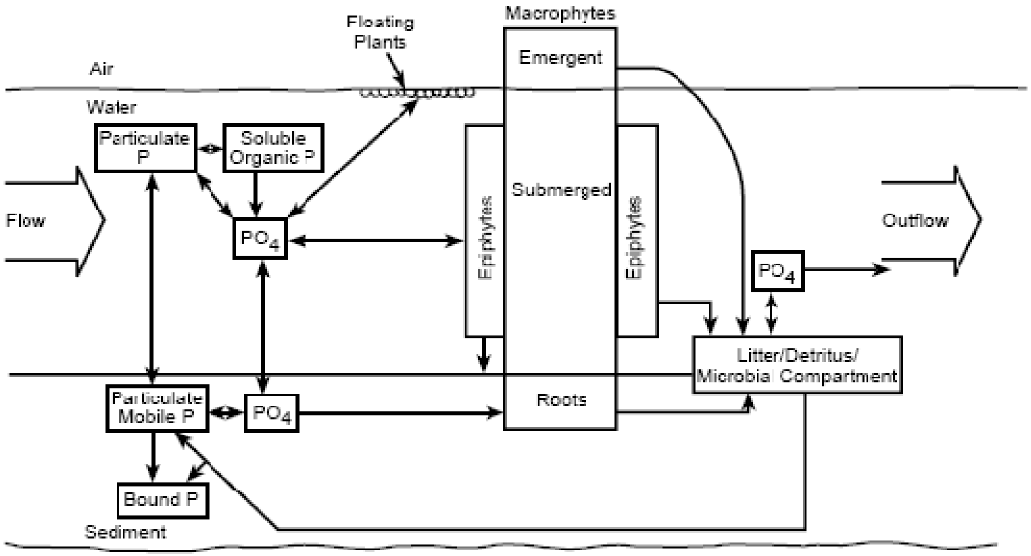

Figure 2.3: Phosphorus transformations in a FWS wetland (EPA,1999).

Phosphorous removal in wetland treatment systems occurs from adsorption, plant absorption, complexation and precipitation (Watson *et al.,* 1989).The major removal of phosphorus is done by uptake from plant roots. The absorption through leaves and plant parts are usually very low and thus macrophytes account for most of removal at the beginning of the growing season. The storage of phosphorus in plants varies between the type of plant and storage below ground is usually longer than storage above ground. Phosphorus is released in portions at varying times throughout the year and is cycled throughout the wetland. Phosphorus is also released after a plant dies and begins to decay. The decaying plant matter above ground release phosphorus into the water while decaying roots secrete phosphorus into the soil. Another important chemical transformation is soil adsorption and precipitation. This process involves soluble inorganic phosphorus moving from the pores in the soil media to the soil surface. With increased clay content the soils adsorption qualities increase. Adsorption and retention of phosphorous in wetland soils is controlled by the interaction of redox potential, pH value, Fe, Al, Ca minerals and amount of native soil phosphorous (Lindsay, 1979; Faulkner & Richardson, 1989; Richardson & Vaidyanathan, 1995).

The problem by physical and chemical removal in wastewater wetlands is that the wetland only accounts for storage in soil media or in plant tissue. Plants absorb phosphorous through their roots and transport it to the growing tissues. Each of these will eventually reach capacity and phosphorus removal will cease until the two are replaced. The other mechanism for removal of phosphorus is by biological means but this process still does not allow for much storage. The uptake of phosphorus by microorganisms is rather fast because bacteria, fungi, and algae are able to multiply quickly. The drawback is that they are unable to store large amounts of phosphorus (Ravinet*et. al*, 2009). The extent at which phosphorus can be removed or stored is dependent on the type of wetland being used. There are limiting ways to remove and store phosphorus. These ways include sorption, storage in biomass, and formation of new soil media. The long term solution to removing phosphorus is through peat/accretion but will only be effective if there is lots of biomass. In order to remove phosphorous from wetlands it is necessary to harvest macrophyte biomass.

Adsorption of phosphorus through soil media is mostly used in VSB wetlands but the type of media will determine how well phosphorus is stored. In FWS wetlands the uptake from free floating macrophytes is more important but these plants must be harvested and replaced to maximize phosphorus removal. Removal of phosphorus by biological means is more of a temporary solution since the phosphorus is released in the water once the organism begins to decay. Typical phosphorus removal is in the 40-60 percent range (Vymazal, 2006). It is important to recognize the processes at work and also realize that wastewater wetlands are not capable of meeting primary phosphorus removal standards.

2.3.6 Mechanism of Pathogen Removal

Major concerns with wastewater wetlands are their ability to remove pathogens of helminthes, protozoans, fungi, bacteria, and viruses. There have been reports that wetlands reduced total coliforms by 57 percent, and fecal coliforms by 62 percent. There have also been reports of 98 percent reduction of giaridia and 87 percent reduction of cryptosporidium. The main processes that are involved with pathogen removal is sedimentation. Sediments of wetlands tend to accumulate as vast amounts of coliforms and bacteria. It has been found that river mud contains 100-1000 more times more fecal coliforms than the surface water. This is also similar to salmonella which 90 percent of it accumulates in sediments. These sediments also give some bacteria the ability to survive longer. Viruses tend to attach to colloidal material which takes longer to settle out and eventually settle out in a loose layer above sediment. This layer can be disrupted from human activity or natural storm events which could cause the pathogens to enter the water column. Another way to filter out pathogens is thought to be through the root structure of plants in wastewater wetlands. In a study done by Mohammad Karim he tested whether sedimentation played a significant role in reducing pathogens in wetlands. He observed that there were not large differences in the amount of fecal coliforms and coliphages in the water column and sediments. The attachment to the root structure played a larger part with these pathogens. The report found that *giardia* and *cryptosporidium* had concentrations two to three times larger in sediments than in the water column. A multispecies wetland showed 73 and 58 percent removal of *giardia* and *cryptosporidium* and a duckweed wetland showed 98 and 89 percent prospectively (Karim *et al*., 2004). Wastewater wetlands offer promise of the possibility of removing pathogens. Removing pathogens from the water table does not mean that pathogens are gone for good. It is possible that pathogens can re-enter the water table but further work must be done on pathogens survivability in wetlands.

2.3.7 Mechanism of Metals Removal

There are some metals that are required for plant and animal growth but these are in very small amounts. These nutrients include copper, selenium, and zinc. These micronutrients are toxic at higher concentrations but other metals can be toxic at even at low concentrations. These metals include cadmium, mercury, and lead which are typically found in industrial wastewater. These toxic metals have no known benefit but can lead to health hazards in humans. Removal of metals in wastewater wetlands occur by plant uptake, soil adsorption, and precipitation. The ability of plants to uptake metals depends on the type of plant and type of metal. There are some types of plants which are capable of storing large amounts of metals in plant biomass and in its roots (DeBusk, 1999b). One such plant is duckweed which is known to store large amounts of cadmium, copper, and selenium. The metals that pass by the root structure tend to accumulate on the structure of the root rather than being absorbed by the plant. Wetland soils are also sources into which can trap metals. Metals including cadmium, copper, nickel, lead, and zinc form insoluble compounds when interacting with sulfides under anaerobic conditions in the soils of wastewater wetlands. This minimizes the ability of these metals to resolubilize under anaerobic conditions. Through a process called chemisorption, metals such as chromium, copper, lead, and zinc form strong chemical complexes with the organic material that is present in the soil and water.

Furthermore, the metals chromium and copper can be chemically bound to clays and oxides and allowed to settle out (EPA, 1999). In a study done by M.A. Maine studied metal uptake in a wetland containing of several plant species. The wetland had 80 percent of cover by *Eichhornia crassipes* (water hyacinth), 14 percent cover by *Typha domingensis* (cattail), and four percent of *Panicum elephantipes* (elephant panicgrass). The wetland removed 86 percent of chromium and 67 percent of nickel. The concentration of zinc was below 50µg/L in most of the samples. Iron sulphide precipitation helped in reducing the iron content by 95 percent. A larger wetland and a smaller wetland were tested where the metals in the larger one were retained by macrophytes and retained by sediment in the smaller wetland (Maine et al., 2006). Wastewater wetlands show promise for removal of metals but further research is needed in this area.

In a study conducted by Rai and Tripathi, 2009 it was found that *Vallisneria spiralisin* was able to remove 70% to 84% of Hg with initial concentration of 0.1, 0.5,1.0 and 3.0 mg/L

2.4Use of wetland vegetation after treatment

a) Typha, brachiaria as fodder

There is a large body of information available on tolerance of forage grasses to salinity, especially those adapted to water logged saline conditions (Russell, 1976; Maas and Hoffman, 1977; Ahmad and Ismail, 1992; Truog and Roberts, 1992; Kumar, 1998; Qureshi and Barrett-Lenard, 1998). Amongst the well known wetland emergent species, *Brachiaria mutica* grass is extensively used as fodder (Katsenovich et al., 2009). Other wetland grasses such as typha, water hyacinth are also popular as fodder. *Brachiaria ramose* is cultivated in south India (Kimata et al., 2000; Porteres., 1976). Some African species of *Brachiaria* have been introduced into the Americas as pasture grasses such as *B. plantaginea* and *B. mutica* (Parsons, 1972). Five species of *Brachiaria* have been released as commercial cultivars in different tropical American countries (Keller-Grein et al., 1996). Currently, the genus *Brachiaria* is the most widely used forage grass in South American savannas due to its physiological tolerance to low fertility acid soils of the tropics (Rao et al., 1996). In a study by Tomar *et al.,* (2002), it was observed that higher productivity of green fodder (19.7-25.7mg/ha) was monitored in case of grass species like *Brachiaria mutica, Panicum maximum,Panicum laevifolium* than other species. The yield was observed to be maximum during July-September period and was low during winter season.

b) Eichhornia in composting

In a study by Anushree (2006), uses of *Eichhornia crassipes* for biogas production, composting, fish feed was documented. Better yield of biogas are obtained using mixture of animal waste and water hyacinth (Kumar, 2000). Pretreatment with fungi or chemicals increases the biodegradeability of water hyacinth for sufficient biogas production (Ali et al., 2004).

Water hyacinth can be used on land either as surface mulch (Woomer et al., 2000) or as compost. Substantial work on vermicomposting of water hyacinth has been conducted in India in terms of optimization of the earthworm species and worm density, pretreatment ofwater hyacinth prior to composting as well as development of high rate vermireactors (Gajalakshmi et al., 2001(a)(b), 2002(a)(b). composting with Eichhornia was found to increase production of lady's finger (67%), potato (14%) and tomato (90%) as compared to control (no mulching) treatment (Sannigrahi et al., 2002).

c) Floriculture (water lily, cana lily etc.)

Canna (or canna lily, although not a true lily) is a genus of nineteen species of flowering plants. The species have a 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. An example of canna liliy being used for floriculture is the reclamation of land next to INA metro station (New Delhi) where the land is turned into a park with only canna lily species.

2.5About *Brachiaria Mutica*

Brachiaria mutica is a C-4 plant that posses Kranz leaf anatomy which is characterized by a bundle sheath of Kranz cells. Within the bundle sheaths, the 4-carbon molecular species transported from the mesophyll is decarboxylated by the appropriate enzyme (Gutierrez et al., 1976). They are perennial and invasive in nature. They are characterised by dense growth, somewhat similar to tanner grass (*Brachiaria arrecta*), but with paired spikelet. They are an easily available specie and ideal for wetland phytoremediation process due to the fact that they due to their dense, intertwined growth provide a mesh sort of filtration, similar to the screening process in conventional treatment process. Moreover the fact that it is highly adaptable makes it a suitable choice for constructed wetland treatment in regions having harsh and hot climate.

2.5.1 Taxonomy

Brachiaria mutica is a creeping perennial grass with long, coarse stolons upto 5.0m, very hairy, decumbent stems and soft, moderately hairy leaves upto 20mm wide and 30 cm long. Leaf sheat has a densely hairy collar. Inflorescence is a panicle 6-30cm long. Stolons and branches root readily at the nodes. It belongs to the family of Poaceae (alt. Gramineae). Its common names are Para grass (Africa, Australia, USA); buffalo grass, Dutch grass, giant couch, Scotch Grass; Mauritius signal grass; herbe de Para (French)

2.5.2 Growth/prescence in different geographical region

This plant is probably native to flood plains of sub-saharan tropical Africa. It is extensively found in swampy plains, marshes and wetland areas. Due to its invasive nature, it spreads in its vicinity. It is planted for grazing in flat, poorly drained or high rainfall environments. It is also used as a cut-and-carry forage. These can be cut for hay but is slow to dry in humid environments where it grows productively

Rested wetland areas can be used a dry season reserves of green feed. A similar system uses shallow water ponding on the edges of which para grass continues to grow as the water recedes. Para will grow in water to 1.2 m deep in the tropics

2.5.3 Growth conditions

The growth condition of *Brachiaria Mutica* depends largely on soil conditions, moisture/water availability, temperature and sunlight. The following are described briefly.

2.5.3.1 Soil requirements

It is well adapted to a wide range of soil types (from sandy to clay soils) of moderate to good fertility. Suited to poorly drained (swampy or seasonally waterlogged) land in the tropics and warmer subtropics, but will also grow productively on free-draining soils in high rainfall environments. Tolerates moderate salinity, low pH to 4.5 and high levels of trace elements normally produced under water-logged conditions.

2.5.3.2 Moisture:

It is found to grow suitably in humid to sub-humid regions with 1,200– 4,000 mm annual rainfall. These can also grow in swampy areas of drier environments down to 900 mm annual rainfall, but will not tolerate extended dry conditions. Para grass can stand long-term flooding. Tolerance of depth of water is probably related to water temperature, as para grass tolerates depths of up to 1.2 m in the tropics but only up to 30 cm depth in the subtropics. Hairy leaves and long hollow stems will float on water, but roots cannot tolerate continuous submergence. They develop adventitious rootlets under flooded conditions.

2.5.3.3 Temperature

It grows in warm seasons only, with growth restricted by temperatures below 15ºC. They are very frost sensitive. The leaves are killed by frost but plants can recover.

2.5.3.4 Light

They are moderately tolerant of shade but prefer full sun. They have lower shade tolerance than signal grass *(B. decumbens*). Hence they are grown under extensive areas of mature coconuts in the Philippines but due to their invasive nature, are prone to weed invasion.

2.5.4 Reproductive development

Reproductive development of para grass is poorly understood. They are reported to be a short-day species that flowers most prolifically in humid environments at latitudes of 10–20º. Dry conditions may stimulate flowering in the subsequent wet season. Adequate soil fertility may also stimulate flowering and seed set. Little or no flowering is reported at subtropical latitudes. In northern Australia, para grass flowers in late April/early May and sets seed in May.

2.5.5 Compatibility (with other species)

In ponded pasture systems in seasonally dry northern Australia, para grass has been grown with grasses that can grow in water to 1.2 m deep; these include aleman grass (*Echinochloa polystachya*) cv. Amity and hymenachne (*Hymenachne amplexicaulis*) cv. Olive. Despite their successful animal production, these 'water' grasses are now regarded as environmental weed because they are capable of invading wetlands.

2.5.6 Ability to spread

They spread rapidly (up to 5 m in a year) into suitably moist soils through its long stolons and possibly through water-borne seed. Para grass has spread throughout the humid tropics following introduction as a pasture grass.

In general it can be said that these are native to swampy, flooded areas. They support moderately high levels of ruminant production. These spread rapidly from stolons. Their young leaves are very palatable and hence can be used as an alternative to fodder. They are frost sensitive and growth is restricted below 15˚C but overall highly adaptable to tropical conditions. In a study by Engel & Parrotta (2001), it was observed that the plant was well adapted to moderately acidic and leached quartz soils of low fertility and subjected to severe erosion. The site was later used for livestock grazing and it was dominated by *Brachiaria decumbens.* This vouches for the ability of *Brachiaria mutica* to adapt to unfavourable conditions, unlike other species and hence is a viable option to be used for treatment of effluent using CW system at such regions.

MATERIALS AND METHODS

The aim of the study was to monitor and study the removal rate and efficiency of phosphates as AP and TP. For this a bench scale *Brachiaria* based constructed wetland cell was prepared and subjected to wastewater. The following methods were employed for the same.

3.1 Study Area (Bench- scale CW Cell)

The system consisted of a bench-scale subsurface flow wetland (SSFW) built in laboratory at Delhi Technological University in which *Brachiaria mutica* was planted and grown. The CW cell had soil substrate packed with gravel-sand mixer and it consisted of a 35cm thick layer of soil substrate. Tanks were of dimension 80 cm wide and 110 cm long with the surface area of tank as 8800cm². The water depth of reactor was kept 5cm above the surface with an effective volume of *400l.* Before starting the study, the wetland system was flushed with water entering from the top and exiting from an outlet provide at the bottom of the system.

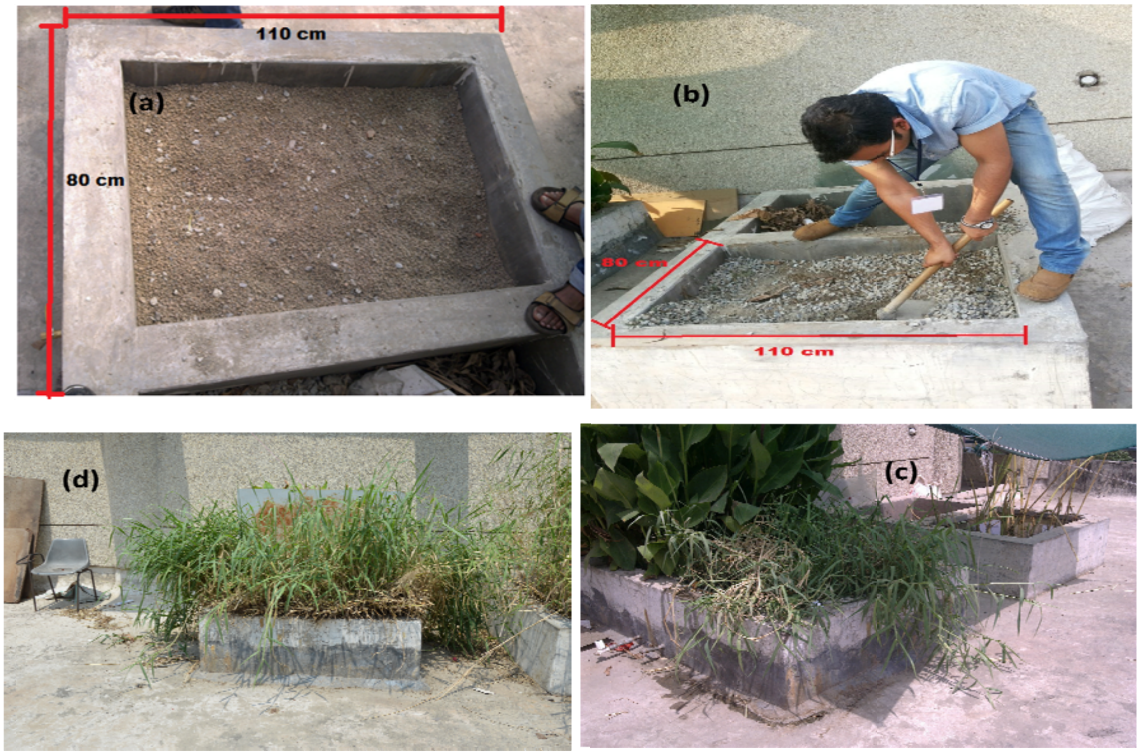

Figure 3.1 Unprepared bed (a); preparation of bed (b); planted *Brachiaria mutica* in CW cell in initial stage (c); stabilised CW cell used during the study (d)

3.2 Selection of Plants

Brachiaria plant was used for the study and it was planted in the bed of the CW. The plant was uprooted from DTU Campus Lake and planted in the CW cell and with average length of 72.14 cm. The plants brought from DTU Lake were already growing and apparently they were healthy. The system was exposed to sunlight and atmosphere and temperature variation was as that found in the natural environment. After the experimental period, the plant had grown quite dense than when it was planted, due to its invasive and expansive property and seemed well adapted to the CW conditions. The length of *B. mutica* increased to approximately 213.44 cm by the end of the study indicating its adaptability to the bench scale CW cell.

3.3 Adaptation of Plant

The plants were planted in the month of April, 2013 after which it received a rainfall and a considerable time was given to the *Brachiaria* to adapt in its new habitat. The system was fed with water at the rate of 20 *l*/day. The system was also fed with fertilizers Di-ammonium phosphate (DAP) and urea, of about 50 g each.

3.4 Design of Experiments

The experiments were designed in the following way to achieve the desired objectives:

(i) Preparation of Synthetic Waste Water

The waste water was synthetically prepared in the laboratory using analytical grade (AG) potassium di-hydrogen orthophosphate salt (KH_2PO_4). The waste water so made had phosphates (PO_4^{3-}-P) in varying concentrations which were 5 mg/l; 10 mg/l and 20 mg/l. Ammonium ions (NH_4^+) were also added to check CW cell's nitrification. Phosphate and ammonium ions were then analysed using the standard methods.

(ii) Effect of Influent Concentration

The water which was fed to the plant had phosphate PO_4-P concentration which varied from **5 ppm** at the initial stage; followed by **10 ppm**;and finally **20 ppm** during theperiod of study.The increase in concentration of nutrients in such a phased manner was to firstly get the plant adapted to the dosage, starting from a low to high concentration, as a sudden exposure to high concentration of nutrients might lead to a high nutrient chemical shock on the plant causing detrimental effect. Secondly, a gradual increase on concentration would allow us to have a better picture of plants adaptability and response to nutrient increase, marked affect of increase in concentration in removal efficiency, plants health etc. Different concentration of ammonium ions NH_4^+ were also added in the form of synthetically prepared waste water when the influent phosphate concentration was **10 mg/l**, ammonium ion concentration was **50 mg/l** and followed by ammonium ion concentration of **100 mg/l** when phosphate was **20mg/l.**The ratio of **N:P** was kept **5:1** for inlet which means, for phosphate concentration of **10 mg/l**, nitrate concentration was **50 mg/l**. The ammonium ions were added to the CW system in order to take care of the nitrogen deficient nitrification conditions.

(iii) Effect of Seasons

The study was mainly aimed at monitoring the growth and phosphate removal efficiency of *Brachiaria*. Phosphate removal efficiency is determined by the nutrient uptake capacity of the plant and the uptake capacity of the plant in return depends upon the meteorological conditions since more is the sunshine hours, more will be the photosynthesis process, more will be the metabolic activities and thus more will be the uptake of phosphates from the waste water. For the purposes described above, different seasons studied were autumn, winter, spring and summer season. The effect of rainfall was also considered in the study. The CW cell was constructed over the roof without shade to study the efficacy of plant under natural conditions.

(iv) Redox Conditions

The reduction and oxidation (redox) processes in the CW cell which takes place were monitored using nitrifying conditions and ferric to ferrous ratio in effluent.Ammonium ions (NH_4^+) were added to the CW cell from the outside in the form of waste water and it was analysed for the nitrification process. Due to nitrification, ammonium ion gets converted to nitrites (NO_2^-), which on its nitrification becomes nitrates (NO_3^-) which is an oxidation process.Total dissolved iron (TDI) and ferrous (Fe^{2+}) ion was analysed for the effluent and from TDI and ferrous ion concentration, ferric (Fe^{3+}) ions concentration was analysed. The ratio of ferric ion to ferrous ion (Fe^{3+} to Fe^{2+} ratio) tells whether the reaction is reducing or oxidising (redox conditions).

3.5 Analysis: Waste Water Analysis

During the experiment the flow rate had been taken constant and Hydraulic Retention Time (HRT) was 24 hours. Samples of treated water were collected from the wetland cell after every 24 hours (HRT-1 day) for 7 months during the study. The samples were tested for the fractions of Phosphate concentration which are Available Phosphate (AP) and Total-Phosphate (TP) using standard method as prescribed by APHA (1997). The concentration of ammonium ions (NH_4^+) and organic nitrogen present in treated waste was determined by Kjeldahl's method.

(a) Characterisation of Phosphate

The phosphate is generally considered as the critical nutrient for the growth of algae in water. The enrichment of this nutrients leads to the process of eutrophication. The most important sources of phosphates are the discharge of domestic sewage, detergents and agricultural run-off.

Available Phosphate

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

Chemicals required were ammonium molybdate and stannous chloride. After acid digestion of samples, ammonium molybedate of around 2mL was added to 50mL of sample and mixed well. This was allowed to stand for a minute and then one drop of stannous chloride was added to this sample. A reaction took place leading to formation of phospho-ammonium molybdate complex which resulted in a blue colour formation in presence of phosphate. In abscence of phosphate, blue colour did not develop, as was in the case of distilled water. The intensity of blue colour developed was directly proportional to the concentration of phosphate. In case of deep blue colour the sample had to be diluted for correct reading. The final recording was then multiplied with the respective dilution factor.

The chemicals required for this method were prepared the following way:

1) Ammonium Molybdate

In a flask, 25 gm of Ammonium Molybdate was dissolved in 175 ml of distilled water. In a separate conical flask 280 ml of concentrated sulphuric acid in 400 ml of distilled water was added. The two solutions were mixed to make the final volume 1*L*.

2) Stannous chloride

For preparation of Stannous Chloride solution, 2.5 gm of stannous chloride was dissolved in 100 ml of Glycerol. The solution was heated with intermittent mixing to make a clear viscous solution.

3) Preparation of standards

For preparation of standards, 0.143 gm of potassium di-hydrogen phosphate was mixed in 1*l* of distilled water to obtain 100 ppm of phosphate stock standard solution. 10 ml of this solution was taken and final volume was made 100 ml to obtain 10 ppm of phosphate standard solution.

Serial dilutions were used to prepare 10 standards in the range of 0.1 to 1.0 ppm. Distilled water was used as the blank, which has zero phosphate concentration.

Preparation of Standard curve

1) To 50 ml of 0.1 ppm phosphate standard, 2 ml of ammonium molybdate was added and mixed well.
2) Later, 5 drops of stannous chloride was added to it and mixed well.
3) The mixture was allowed to stand for 5 minutes; blue colour appeared in this solution.
4) Absorbance was noted at 690 nm on spectrophotometer.
5) The above steps were repeated for 0.2ppm, 0.3ppm, 0.4ppm, and so on till 1ppm.
6) Graph was plotted between concentration and absorption. Best fit line was drawn.

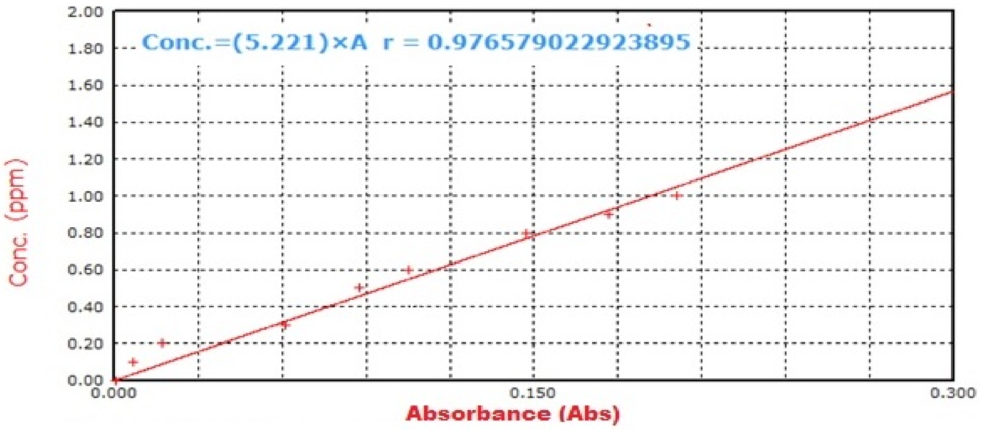

Figure 3.2: Standard curve for phosphates

(Labtronics make Model LT-290 spectrophotometer)

Analysis of available phosphate

1) To 10 ml of sample, 0.4 ml of ammonium molybdate was added and mixed well.
2) To this, 2 drops of stannous chloride was added and mixed well.
3) The mixture was allowed to stand for 5 minutes; blue colour appeared in this solution.
4) Absorbance was noted at 690 nm and 100% transmittance on spectrophotometer.
5) Multiplication of absorbance with graph factor gave the phosphate concentration of sample.
6) Care should be taken that absorbance is measured after 5 minutes after adding stannous chloride and before 11 minutes. It should also be ensured that spectrophotometer has been switched on 30 minutes before the absorbance is noted. The blank sample should be set to zero before sampling and should be checked in between alternate samples to ensure there is no deviation.

Total Phosphate

Total Phosphate was extracted by Sulphuric acid-Nitric acid digestion method. The acids i.e. conc. Sulphuric acid and conc. Nitric acid plays the role of converting bound phosphates in unbound form. Thus all phosphate present in acid digested sample is in unbound form which can later be directly read from spectrophotometer using ammonium molybdate - stannous chloride method.

Chemicals Required

1)Concentrated Sulphuric Acid (H_2SO_4)

2) Concentrated Nitric Acid (HNO_3)

Apparatus Required

A Micro wave with six digestion units at least.

Extraction of Phosphate (Acid digestion)

The sample collected is taken in the different micro wave digestion units and to every unit, add acids so that the phosphate bound with iron, calcium and aluminium will become unbounded and shall make the total phosphate in the water sample during the study. The acids are added in the ratio of 1:3 (Nitric Acid: Sulphuric Acid)

Analysis of Total Phosphate

1) To 10 ml of sample, 0.4 ml of conc. H_2SO_4 was added with 2ml of conc. HNO_3.

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

3) After completion of digestion, sample was neutralised with NaOH. Neutralisation was seen as pink colour which appeared after adding 2 drops of phenolphthalein before adding NaOH solution to the digested samples.

4) To 50 ml of sample, 2ml of ammonium molybdate was added, and mixed well.

5) To this, 5 drops of stannous chloride was added and mixed well.

6) The solution was allowed to stand for 5min, blue colour appeared in the mix.

7) Absorbance was noted at 690 nm on spectrophotometer

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

9) Care should be taken that absorbance is measured after 5 minutes after adding stannous chloride and before 11 minutes. It should also be ensured that spectrophotometer has been switched on 30 minutes before the absorbance is noted. The blank sample should be set to zero before sampling and should be checked in between alternate samples to ensure there is no deviation.

(b) Total Kjeldahl's Nitrogen (TKN)

The complicated nitrogen cycle found in CW system is attributed to a number of factors that influences the transformation of this element during wastewater treatment. The most important soluble inorganic forms of nitrogen are ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) Total Kjeldahl's Nitrogen process was used for finding out the organic nitrogen as well as ammonia in the waste water sample. The summation of all inorganic and organic forms is referred to as total nitrogen (TN). TN variations were closely monitored during study. This was done using a micro Kjeldahl's unit.

Description of Apparatus:

Micro-TKN apparatus consisted of the following units:

Boiler/ Steamer:

It produces the steam with the heat coming from the heating mantle over which it is placed.

Steam Trap:

It traps the excess steam and also collects the waste water during the back wash of the apparatus.

Digestion and Distillation Unit:

In this unit, the digestion and the distillation of the sample take place and the distillate goes to the next unit.

Condenser Unit:

It condenses the distillate coming out from the digestion unit and then it is collected and then the sample is titrated against hydrochloric acid.

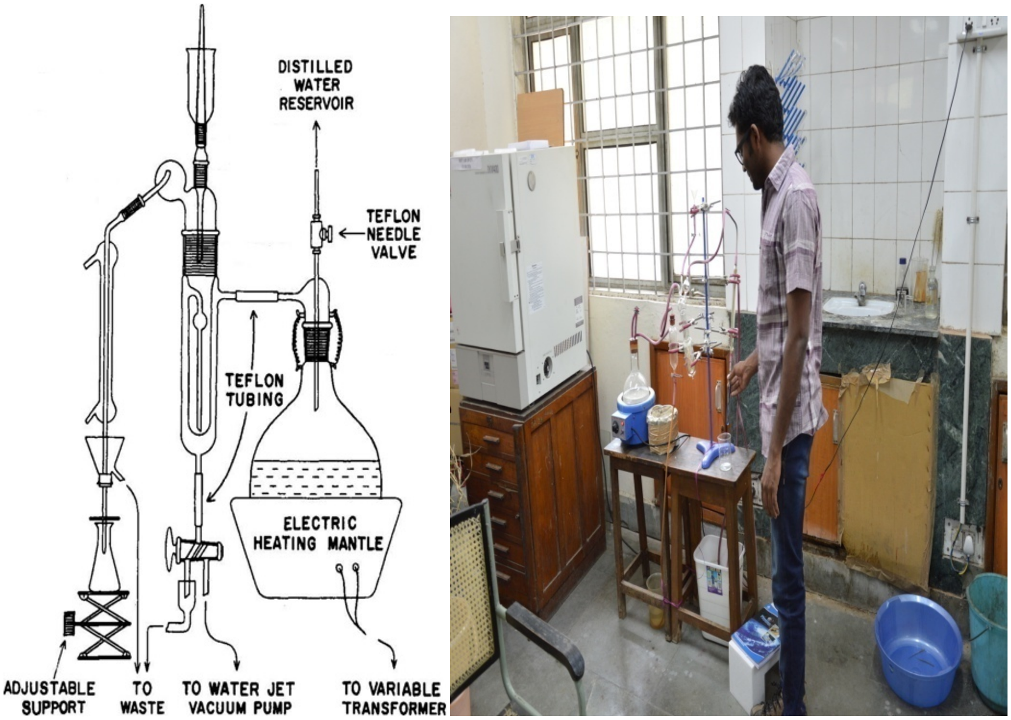

Figure 3.3: Micro Kjeldahl apparatus

Preparation of chemicals required:

1) Borax buffer, 4%:

To 100 ml of distilled water, 4 g of borax $Na_2B_40_7$ was added and heated to mix the crystals well.

2) Boric Acid and Mixed Indicator:

To 100 ml of distilled water, 4 g of boric acid was added. To 100 ml of boric acid solution, 5 ml of mix indicator was added and mixed well.

Mixed indicator was boric acid + mixed alcohol solution of bromocresol green (0.5%) and methyl red (0.1%) in 2:1 ratio. In case solution turns bluish, 0.01 N HCl was to be added until the colour just turns pink to brown.

3) Titrant:

0.01N HCl was used for the titration. 1 N HCl solution was prepared and then diluted in series.

Analysis of Total Kjeldahl's Nitrogen

1) The boiler/ steamer was heated to get the steam.

2) 10 ml of the sample was taken in a rinsed test tube.

3) To the 10 ml sample, 1 ml of the borax was added.

4) In another test tube 5 ml of the boric acid with mix indicator was pippetted.

5) The sample and borax mix was allowed inside the distillation and digestion unit of the apparatus as soon as the boiler started producing steam.

6) Without any lapse in time, test tube containing boric acid with mix indicator was placed at the open end of the condenser unit and nitrogen was collected in test tube. Dark green to blue colour appeared in test tube depending on intensity of nitrogen in the sample.

7) The nitrogen sample thus collected was then titrated until the colour changes from blue to orange.

8) The volume of the titrant consumed was noted down.

Calculations:

$$\boxed{\textbf{TKN (mg/l)} = \frac{\textbf{(Volume of titrant in ml) x (normality of titrant) x 14 x 1000}}{\textbf{(Volume of sample taken in ml)}}}$$

(c) Iron

Iron is present in the sediments of the bed of the wetland. The fractions of iron studied here are total dissolve iron (TDI) and ferrous. Consequently ferric was calculated. This was found out using **Phenanthroline Method.**

Reagents:

Use reagents low in iron. Use iron free distilled water in preparing standards and reagent solutions. Store reagents in the glass stopper bottles. The HCl and ammonium acetate solution are stable for indefinitely if stoppered tightly. The hydroxylamine and phenoanthroline solutions are stable only for few months.

1) Hydrochloric acid:

ConcentratedHCl containing less than 0.00005 % iron

2) Hydroxylamine solution:

10g of hydroxylamine $NH_2OH.HCl$ was dissolved in 100 ml water.

3) Sodium acetate solution:

100g of sodium acetate $NaC_2H_3O_2.3H_2O$ was dissolved in 800 ml water.

4) Phenanthroline solution:

100 mg 1, 10-phenanthroline monohydrate $C_{12}H_8N_2.H_2O$ was dissolved in 100 ml of water by stirring and heating to 80^oC. Care shold be taken that the solution does not boil. Heating is unnecessary if 2 drops of conc. HCl is added to the water. Solution is to be discarded if it darkens.

5) Preparation of standard iron solution

0.0496 g of $FeSO_4.7H_2O$ was mixed in 100 ml of distilled water to get 100 ppm concentration iron solution. 10 ml of this 100 ppm iron standard solution is taken and volume was made up to 100 ml to get final 10 ppm of iron concentration standard solution. Series dilution was done to get the standard solution from range 0.1 ppm to 1 ppm.

Preparation of the Standard Curve:

1) 5 ml of the standard solution was taken in a 50 ml volumetric flask.

2) To the 5 ml standard, 1 ml of hydroxylamine was added and shaken well.

3) To the previous solution 5 ml of the sodium acetate solution was added and shaken.

4) To the previous solution 5 ml phenanthroline solution was added and mixed well. Red or orange colour developed.

5) The volume of the solution was made 50 ml by using distilled water.

6) This sample wass allowed to stand for 10 minutes.

7) The absorbance of the solution was noted at 510 nm wavelength and 100 % transmittance.

8) The above procedure were repeated for the rest of the standards also.

9) The plot between concentration and absorbance was drawn. The best fit line was taken.

Figure 3.4: Standard curve for iron
(Labtronics make Model LT-290 spectrophotometer)

Analysis of Total Dissolved Iron (TDI)

1) 5 ml of the sample solution was taken in a 50 ml volumetric flask.

2) To the 5 ml standard, 1 ml of hydroxylamine was added and shaked for mixing.

3) To the previous solution 5 ml of the sodium acetate solution was added and mixed well.

4) To the previous solution 5 ml phenanthroline solution was added and mixed well. Red orange colour developed in this mix.

5) The volume of the solution was made up to 50 ml by using distilled water.

6) The sample thus obtained was allowed to stand for 10 minutes.

7) After that the absorbance of the solution was noted at 510 nm wavelength and 100 % transmittance.

8) Graph factor was multiplied with the absorbance to get the concentration of the total dissolved iron.

9) A blank was also prepared using distilled water.

Analysis of Ferrous (Fe^{2+}) ions:

1) 5 ml of the sample solution was taken in a 50 ml volumetric flask.

2) To the previous solution 5 ml of the sodium acetate solution was added and mixed well.

4) To the previous solution 5 ml phenanthroline solution was added and mixed well. Red to orange colour developed in this mix.

5) The volume of the solution was made up to 50 ml by using distilled water.

6) The sample thus obtained was allowed to stand for 10 minutes.

7) After that the absorbance of the solution was noted at 510 nm wavelength and 100 % transmittance.

8) Graph factor was then multiplied with the absorbance to get the concentration of the ferrous.

Analysis of Ferric (Fe^{3+}) ions:

From the known concentration of TDI and ferrous (Fe^{2+}),concentration of ferric (Fe^{3+}) can be estimated. Ferric concentration is difference in concentration between TDI and ferrous (Fe^{2+}).

RESULTS AND DISCUSSION

Brachiaria mutica plant was planted in Constructed Wetland cell, well before the study was to be conducted so as to provide ample growth time for the plant to adapt to the new environment, while all measurements were to be performed later on. With reference to this study, the readings and measurements were noted from October 2013 to April 2014. During the study, average temperature ranged from 10˚C to 32˚C and rainfall events were also noted.

The study aimed to monitor the removal efficiency of *Brachiaria mutica* for the phosphates in wastewater during different seasons and at different loading rates. The effect of increasing concentration of phosphate on the removal efficiency, and plant health were also studied. Monitoring of redox conditions in CW cell was also done based on oxidation of ammonium (NH_4^+) ions and ferric to ferrous ratio. During the study, pH, electrical conductivity (EC) and total dissolved solids (TDS) were also monitored regularly to understand any other change in the system. Meteorological parameters were also monitored to study the effect of seasons on removal behaviour. The results so obtained are given below.

4.1 General Observation

As per the general observations it was observed that phosphate removal efficiency varied differently for different seasons. It was observed that phosphate removal efficiency was minimum during winter season, whereas, it was maximum during summer season. It followed the trend as winter season < spring season < autumn season < summer season, having the total phosphate removal efficiency (%) of the order 55.24 < 78.8 < 80.7 < 85.6, respectively. During the study, the efficiency was low in the winter season because the system was fed with only one type of nutrient which caused excessive loading of phosphates (PO_4^{3-}-P), and deficiency of nitrogen primarily fed into the cell externally. The plant variant is also not adapted to cold condition, which is assumed as another reason for the low removal efficiency (Tomar *et al.*, 2002; Kaseva 2004; Bojcevska & Tonderski, 2007; Katsenovich *et al.*, 2009). TDS level for the outlet has a significant difference in its concentration at the beginning and at the end of the study and the TDS level ultimately increased. Electrical Conductivity (EC) of the system also showed an increasing trend and reached its maximum at 3960µS/cm in summer season. It is noted that EC increased for both inlet and outlet. Ammonium ions added to the CW cell also got converted to nitrates (NO_3^-) owing to nitrification by micro organisms and were subsequently taken up by the plants.

Performance of CW cell

The monitoring of the CW occurred over the course of 07 months (October 2013 to April 2014). During this period, the CW performance was assessed daily by monitoring the concentration of phosphate in influent and effluent at a HRT of 24 hours. Water quality monitoring of the effluent was confined to phosphate only for this study and adhered to EPA methods (APHA, 1995). The analysis was performed in triplicates using AR grade chemicals.

The targeted hydraulic loading rate of 4cm/day and maintained a surface water depth of 12cm. Mean hydraulic rate varied between 3.8 to 4.4cm/day by adding a volume of approximately 16L of influent. During winters however the hydraulic rate was reduced to about half of summer, i.e. approximately 8L of influent, due to lower evapotranspiration by plants and lower rate of evaporation.

4.2 Meteorology of Study Area

Meteorology of study area for the present report is confined to ambient temperature profile and rainfall conditions which are described as follows.

4.2.1 Ambient Temperature Profile

The ambient temperature profile for the period of 07 months was studied from October, 2013 to April, 2014 (ANNEXURE-I, Figure 4.1). The effect of ambient temperature on growth of *Brachiaria mutica* and also on nutrient removal efficiency of the CW cell was studied. The average sun shine hours in a day varied on an average between 8 hours to 10 hours during the study.

Temperature is a primary determinant of nearly all CW parameter outcomes, and the year round warm weather typical of tropical climates has shown to significantly enhance CW performance relative to more temperate regions (Nahlik & Mitsch, 2006; Bojcevska & Tonderski, 2007; Kaseva, 2004).

Measured average temperatures fell in the range of 10˚C to 32˚C from winter to summer season respectively. The plant was seen more adapted to dry and warm conditions than cold conditions. Shade was also provided to the plant but it was observed to grow well during the summer season under natural condition, without shade.

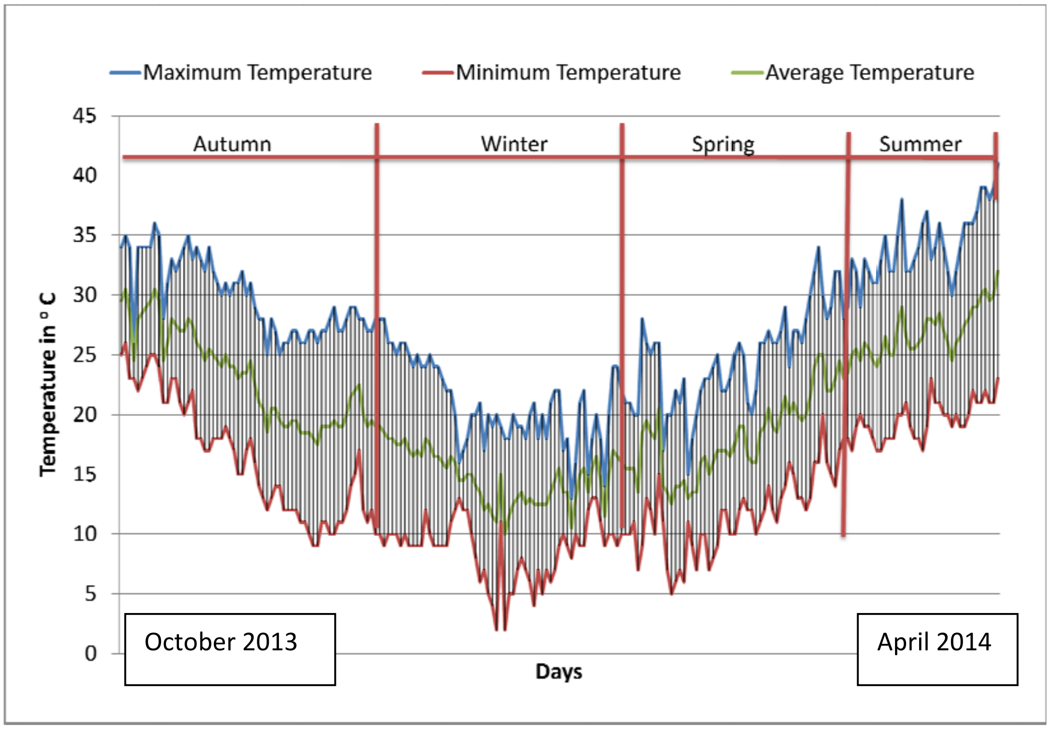

Figure 4.1: Temperature profile during the study

4.2.2Rainfall

Rainfall during the study period was very sparse with only 18 rainfall events. The period of maximum rainfall events was February to March. Rainfall varied in the range of 0.1cm-38cm.

4.3 Sediment Analysis

Soil used for preparation of bed had a specific gravity of 2.74 with a void ratio (e) of 0.77 and bulk density of 17.82. The soil sample had a mixture of 39% gravels, 59% sand and silt (Table 1, Figure 4.2). The soil structure mimicked the type found generally at wetlands. Percolation of influent was easier and easy exchange of gases was possible. Gravels formed the bed of the substrate so that clogging of collection point can be avoided. The soil structure facilitated growth of plant with easy root penetration and exchange of gases was possible.

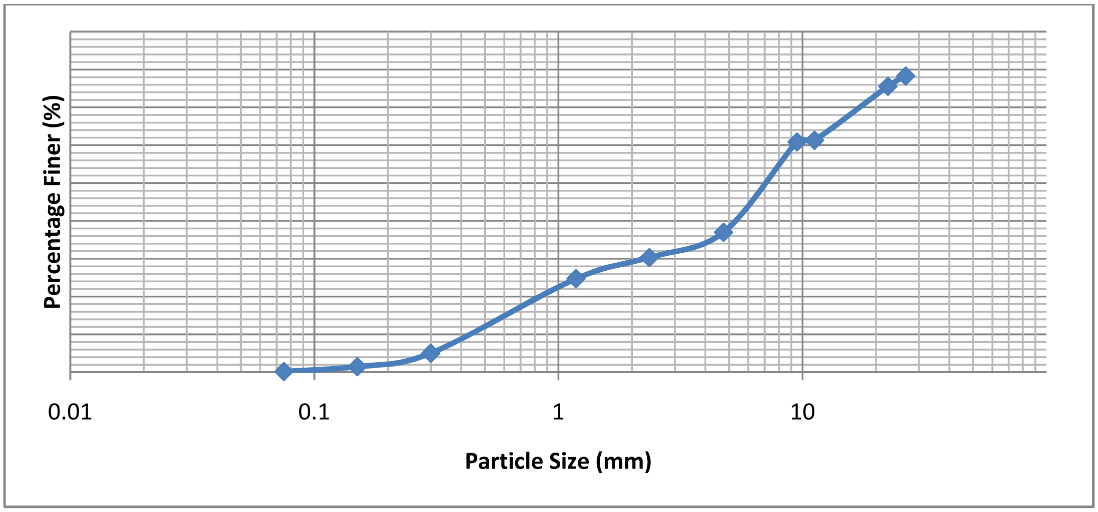

Figure 4.2: Particle size analysis of soil used

Table4.1: Particle size analysis of sediments

Sieve size (mm)	Weight Retained (g)	Percentage Retained	Cumulative Percentage	% Finer
26.5	331.85	21.71	21.71	78.29
22.4	41.52	2.72	24.42	75.58
11.2	218.08	14.26	38.69	61.31
9.5	6.76	0.44	39.13	60.87
4.75	365.28	23.89	63.02	36.98
2.36	101.62	6.65	69.67	30.33
1.18	85.7	5.61	75.27	24.73
0.3	300.84	19.68	94.95	5.05
0.15	54.51	3.57	98.52	1.48
0.075	19.78	1.29	99.81	0.19
Pan	2.9	0.19	100	-
Total	1526.23	100	-	-

4.4 Wastewater Analysis

4.4.1 pH

pH is an important regulatory parameter and must be maintained within range. pH of influent was observed as an average of 7.6 varying in range of 6.7 to8.3. The pH of the inlet was lower initially, at an influent phosphate concentration of 5mg/L. On addition of ammonium sulphate salt, the pH increased to around 8. This change in pH was reflected in the outlet also; however the change was marginal. The pH of outlet ranged from 6.6 to 7.5, the average pH during the study was being 7.2. It was observed that the sediments and plants in the wetland system tend to remove the dissolved nutrients and salts to turn the water to almost neutral. Some degree of algal bloom was observed at the later stage of the study at influent PO_4^{3-}concentration of 20mg/L. It was concluded in the study that the CW had a tendency to bring the pH to neutral even though influent was either slightly acidic or slightly basic. The CW cell thus acted as a buffering system to maintain the pH to almost neutral range, irrespective of the variation in pH, and therefore, can absorb shocks in respect of pH of influent.

4.4.2 Electrical Conductivity (EC)

The average electrical conductivity (µS/cm) of influent went on increasing from 669µS/cm to 933µS/cm to 1408 µS/cm with an increasing concentration of phosphate and ammonium ions from 5 mg/l to 10 mg/l and 50 mg/l; and 20 mg/l and 100 mg/l, respectively. The average EC of effluent varied from 2025µS/cm (at 5 mgP/l) to 1918µS/cm (at 10 mgP/l), to 2496µS/cm (at 20 mgP/l). The average TDS of effluent varied from 989mg/l (at 5 mgP/l) to 865 mg/l (at 10mgP/l) to 1696mg/l (at 20mgP/l). The average value of TDS of varied as 1133mg/l during autumn, 841mg/l during winter, 865mg/l during spring and 1694mg/l during summers. There's a direct relation of TDS with EC. It was observed that EC, and accordingly TDS, was lower during winter season. This was also observed in a study by Kyambadde *et al.,* (2004). However on addition of higher concentration of nutrients in influent, TDS and EC increased in effluent also. It was also observed that TDS and hence EC was higher in warmer season such as autumn and summer. Under tropical conditions evapotranspiration and precipitation are known to exert a substantial effect on CW processes and effluent water quality (Kadlec and Knight, 1996). During dry season, water deficit leads to significant increases in conductivity values and mineralization system. TDS in effluent may be attributed to dissolution of salts from the sediments in lieu of the nutrients removed from the wastewater. The data of electrical conductivity can be used to determine the dilution factor needed for process effluent water for irrigation purposes (Fink and Mitsch, 2004).

4.4.3 Plant growth and health

Plant appearance and health was evaluated over the course of study. Care was taken such that all plants selected for the study purpose appeared healthy, with no indication of any sort of pathology. The plants had developed strong root systems within the wetland soil and were uniform in growth. It was observed that the plant had a good amount of biomass and due to its expansive and invasive nature, it needed trimming. However the plant was allowed to grow without any trimming so that full potential of biomass may be utilised in nutrient removal and also to replicate the natural environment of the plant in a natural wetland where it grows uninhibited. The plant was difficult to maintain due to its haphazard growth, however such a growth is beneficial in wetland since it helps in proliferation of new roots and shoots in neighbouring areas. It is capable of surviving and proliferating in extreme tropical climate as that typical of Africa, Australia etc where the plant is native to. The only cause of concern was during the winter period when the plant showed a retarded growth with a seemingly drying up of tissues and leaves.

4.5 Nutrient Removal Study

The aim of the study was to observe the removal of phosphate by the *Brachiaria mutica* based CW system and to know its removal efficiency. For this the period of observation was divided into different seasons to get an idea of effect of meteorological conditions during different seasons. The nutrients present in waste water are taken up by the plants for their metabolic activities. The chemical forms of nutrients present in the water keep transforming depending on the environmental conditions. There occurs bio-chemical transformation of nutrients. Out of the two forms of phosphate studied, available phosphate (AP) was available to the plants and is taken up readily. Ammonium ions fed into the system are converted by microbes by nitrification to form of nitrites (NO_2^-) which are further oxidised to nitrates (NO_3^-) and NO_3^- are taken up by the plants. The effect of environmental conditions (seasons) on growth and removal efficiency of the plant is given below.

4.5.1 Seasonal Variation

Seasonal variations from October, 2013 to April, 2014, were studied for the removal efficiency of phosphate (PO_4-P) in varying concentrations for its various fractions. Later, ammonium ions (NH_4^+) in varying concentrations were also added to the wetland system. Various seasons studied during the study were autumn, winter, spring and summer in chronological order.

Seasonal effects on vegetative uptake of phosphate over the entire duration were assessed. During higher temperature, marked increase in removal efficiency was noted. During rainy days also there was a reduction in nutrient measured in the effluent, although this was mainly assumed to be due to effect of dilution.

Available and total phosphates are the fractions of phosphate present in any environmental matrix. Waste water was synthetically prepared for inlet and the treated water after a retention period of 24 hours as outlet from the CW cell was analysed. Available and total phosphate were analysed for both inlet and outlet.

4.5.1.1 Autumn Season

The study was initiated in late October 2013 to investigate the removal of phosphate by *Brachiaria*-based CW cell. The initial concentration of TP in synthetic wastewater, was 5mg/l. The solution was prepared in tap water and was analysed for initial concentration of TP. The concentration of TP in influent varied from 2.4mg/l to 3.6mg/l with an average value of 3.2mg/l. Available phosphate (AP) in synthetic waste water varied from 1mg/l to 1.3mg/l with an average of 1.1mg/l. The effluent obtained after an HRT of 24 hours had AP concentration varying from0.11mg/l to 0.20mg/l with an average of 0.15mg/l. Concentration of AP in influent and effluent represented removal efficiency varying from 83.4% to 90.7% with an average removal efficiency of 86.4% during the autumn season. The concentration of TP in effluent varied from 0.27 mg/l to 0.91mg/l with an average value of 0.63mg/l.

Concentration of TP in influent and effluent represented removal efficiency varying from 72.6% to 89.2% with an average removal efficiency of 80.7%. The average removal rate was observed to be 58.48mg/m^2-day; ranging from 43.91mg/ m^2-day to 64.53mg/ m^2-day for TP for average inlet and outlet loading rate of 58.69mg/m^2-day and 0.21 mg/m^2-day (Table 4.2, Figure 4.3).

It was observed that the removal efficiency of *Brachiaria* for AP was slightly higher than that of TP. The reason maybe ascribed to bio availability of AP compared to TP since TP is bound to different elemental entities and organic matter present in sediments and wastewater, its availability to the wetland vegetation is slightly less. The biotransformation of different chemical forms of phosphate results in the conversion of TP to AP. pH of the system, redox conditions and presence of metals (Ca, Mg, Al, Fe etc) regulate the bio chemical conversion of phosphate. The results in the present study represents that conversion of non available/bound phosphate takes place to available phosphate, followed by the uptake by wetland vegetation preferably. The removal efficiency for phosphate went on decreasing from October to first week of December. This might be because of binding to sediments, high initial intake by plants and microbial uptake during the initial phase. The other reason could be a decrease in average ambient temperature from October to December the average temperature decreased from 29.5˚C (October 2013) to 17.5˚C in first week of December 2013. The decrease in ambient temperature results in a decrease in metabolism of plant (Kasev., 2004). A decrease in average sunshine hours was also observed with the season transforming from autumn to winter. A decrease in average sunshine hours results in reduced photosynthesis, decreased productivity and hence reduced uptake of nutrients. The decrease in temperature also results in reduced evapotranspiration and therefore reduced rate of updraft of water through the plant. This too reduces removal efficiency of wetland vegetation for different nutrients (Bojceveska & Tonderski; 2007). Another reason for reduced removal efficiency of *B*rachiaria in later period of autumn season could be limitation of Nitrogen (N) as nutrient. Since there was no external input of nitrogen into the system, the plants faced nitrogen deficiency and it resulted in reduced metabolic rate of *Brachiaria* (Katsenovich *et al.,* 2009).

Table 4.2: Concentration of phosphate PO_4^{3-}-P (mg/l) and its removal efficiency (%) during autumn season

Date (n=30)	Available Phosphate			Total Phosphate		
	Influent (AP_i)* (mg/l)	Effluent (AP_o)* (mg/l)	Removal Efficiency (%)	Influent (TP_i)* (mg/l)	Effluent (TP_o)* (mg/l)	Removal Efficiency (%)
22-10-2013	1.1	0.10	90.7	3.02	0.34	88.7
23-10-2013	1.17	0.11	90.6	3.11	0.35	88.7
24-10-2013	1.15	0.12	89.6	2.42	0.27	88.8
25-10-2013	1.14	0.13	88.6	3.38	0.41	87.9
28-10-2013	1.13	0.12	89.3	3.44	0.37	89.2
29-10-2013	1.02	0.13	87.1	2.88	0.36	87.5
30-10-2013	1.07	0.14	87.2	3.24	0.43	86.7
31-10-2013	1.1	0.15	86.7	2.98	0.45	84.9
01-11-2013	1.13	0.13	88.2	2.88	0.41	85.8
04-11-2013	1.15	0.14	88.1	3.56	0.57	84.0
05-11-2013	1.02	0.17	83.4	3.38	0.81	76.0
06-11-2013	1.13	0.19	83.5	3.44	0.76	77.9
07-11-2013	1.1	0.18	83.5	3.18	0.71	77.7

08-11-2013	1.07	0.18	83.6	3.24	0.72	77.8
11-11-2013	1	0.12	88.0	3.45	0.65	81.2
12-11-2013	1.13	0.19	83.5	3.56	0.76	78.7
13-11-2013	1.19	0.18	84.7	3.11	0.72	76.8
14-11-2013	1.12	0.19	83.4	3.19	0.77	75.9
15-11-2013	1.09	0.18	83.9	3.33	0.79	76.3
21-11-2013	1.15	0.13	89.0	3.08	0.57	81.5
22-11-2013	1.11	0.18	84.1	3.29	0.68	79.3
25-11-2013	1.02	0.13	87.5	3.38	0.73	78.4
26-11-2013	1.09	0.16	85.7	3.15	0.73	76.8
27-11-2013	1	0.16	84.4	3.25	0.77	76.3
28-11-2013	1.2	0.18	85.2	3.23	0.76	76.5
29-11-2013	1.21	0.18	85.0	3.41	0.82	76.0
02-12-2013	1.17	0.12	89.4	3.35	0.63	81.2
03-12-2013	1.06	0.15	86.3	3.31	0.77	76.7
05-12-2013	1.1	0.14	87.3	3.37	0.83	75.4
06-12-2013	1.31	0.20	85.0	3.32	0.91	72.6
Range(Min-Max)	**1-1.31**	**0.10-0.20**	**83.4-90.7**	**2.42-3.56**	**0.27-0.91**	**72.6-89.2**
Mean ± S.D.	**1.114±0.07**	**0.1513 ±0.03**	**86.4 ±0.03**	**3.231 ±0.23**	**0.6283 ±.02**	**80.7 ±0.02**

$^{*}AP_{i}$ = available phosphate at inlet; $^{*}AP_{o}$ = available phosphate at outlet; $^{*}TP_{i}$ = available phosphate at inlet; $^{*}TP_{o}$ = total phosphate at outlet

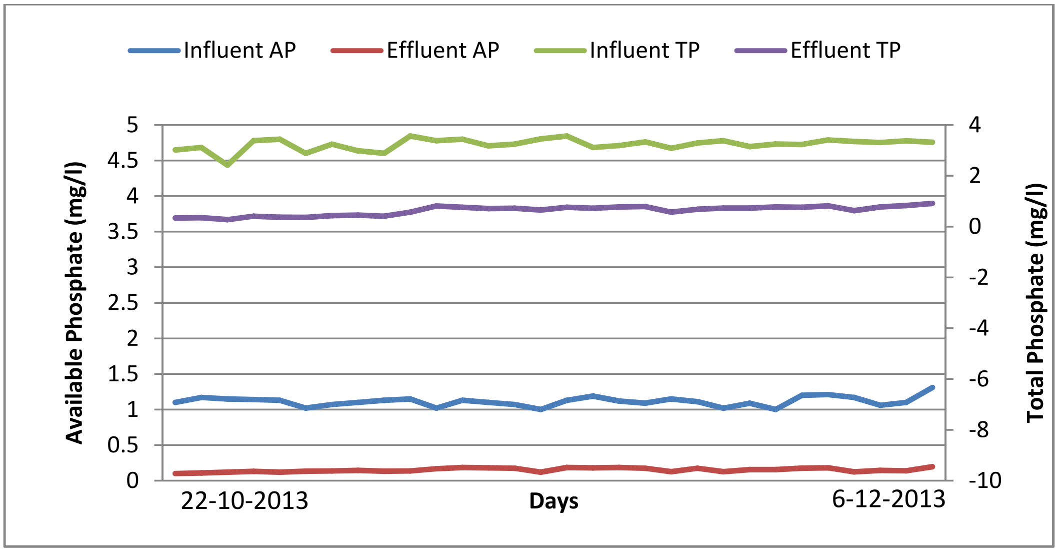

Figure 4.3: Available Phosphate and Total phosphate concentration (mg/l) in influent and effluent in winter season

4.5.1.2 Winter Season

The initial concentration of TP in synthetic wastewater, was 5mg/l. The solution was prepared in tap water and was analysed for initial concentration of TP on regular basis. The concentration of TP in influent varied from 2.39mg/l to 4.72mg/l with an average value of 3.60mg/l. Available phosphate (AP) in synthetic waste water varied from 1.15mg/l to 2.71mg/l with an average of 1.70mg/l. The effluent obtained after an HRT of 24 hours had AP concentration varying from0.43mg/l to 1.53mg/l with an average of 0.70mg/l. Concentration of AP in influent and effluent represented removal efficiency varying from 15.7% to 75% with an average removal efficiency of 59% during the winter season. The concentration of TP in effluent varied from 1.01 mg/l to 2.92mg/l with an average value of 1.55mg/l. Concentration of TP in influent and effluent represented removal efficiency varying from -9.4% to 77.5% with an average removal efficiency of 55.24%. The removal efficiency of -9.4% on 06-01-2014 was due to flushing of nutrients when CW cell was started on first day after the break. The average removal rate was observed to be 64.78mg/m^2-day; ranging from 43mg/ m^2-day to 85.14mg/ m^2-day for TP for average inlet and outlet loading rate of 65.31mg/m^2-day and 0.53mg/m^2-day.

It was observed that the removal efficiency of *Brachiaria* for AP was slightly higher than that of TP. The reason maybe ascribed to bio availability of AP compared to TP since TP is bound to different elemental entities and organic matter present in sediments and wastewater, its availability to the wetland vegetation is slightly less. The biotransformation of different chemical forms of phosphate results in the conversion of TP to AP. pH of the system, redox conditions and presence of metals (Ca, Mg, Al, Fe etc) regulate the bio chemical conversion of phosphate. The results in the present study represents that conversion of non available/bound phosphate takes place to available phosphate, followed by the uptake by wetland vegetation preferably. The average removal efficiency for phosphate decreased from autumn season significantly. The reason could be due to a decrease in average ambient temperature from autumn season to winter season where the average temperature decreased from 23.5˚C in autumn to 12.5˚C in winter. The decrease in ambient temperature results in a decrease in metabolism of plant. A decrease in average sunshine hours was also observed during the winter season. A decrease in average sunshine hours results in reduced photosynthesis, decreased productivity and hence reduced uptake of nutrients. The decrease in temperature also results in reduced evapotranspiration and therefore reduced rate of updraft of water through the plant. This too reduces removal efficiency of wetland vegetation for different nutrients. In a study by Katsenovich *et al.,* (2009), it was observed that CW performance significantly got enhanced on warm weather typical of tropical climate. Similar observation was made by Nahlik and Mitsch (2006) too. The present study gets validated by these. Another reason for reduced removal efficiency of *B*rachiaria in winter season could be limitation of Nitrogen (N) as nutrient. Since there was no external input of nitrogen into the system, the plants faced nitrogen deficiency and it resulted in reduced metabolic rate of *Brachiaria*. As stated earlier, *Brachiaria mutica* has low frost resistance. Hence a decrease in temperature resulted in stress condition for the plant to adapt, thereby adding to the significantly low removal efficiency in winter as compared to the removal efficiency during autumn season, to which the plant was more adapted to. (Table 4.3, figure 4.4)

Table 4.3 Concentration of phosphate PO_4^{3-}-P (mg/l) and its removal efficiency (%) during winter season

Date (n=29)	**Available Phosphate**			**Total Phosphate**		
	Inlet (AP_i) (mg/l)	**Outlet (AP_o) (mg/l)**	**Removal efficiency (%)**	**Inlet (TP_i) (mg/l)**	**Outlet (TP_o) (mg/l)**	**Removal efficiency (%)**
06-01-2014	1.82	1.53	15.7	2.67	2.92	-9.4
07-01-2014	1.66	0.66	60.2	2.39	1.33	44.4
08-01-2014	1.56	1.07	31.5	2.52	1.77	29.8
09-01-2014	1.63	0.97	40.4	2.67	1.82	31.7
10-01-2014	1.66	0.52	68.4	2.89	1.05	63.7
13-01-2014	1.15	0.44	61.8	3.24	1.4	56.7
15-01-2014	1.15	0.44	61.8	3.24	1.4	56.7
16-01-2014	1.65	0.77	53.2	3.67	1.72	53.2
17-01-2014	1.65	0.47	71.5	3.69	1.36	63.2
20-01-2014	1.76	0.67	62.0	3.28	1.33	59.5
21-01-2014	1.80	0.76	57.8	3.00	1.39	53.6
22-01-2014	1.85	0.67	63.7	2.78	1.17	57.9
23-01-2014	1.89	0.64	66.2	2.80	1.11	60.3
24-01-2014	1.43	0.48	66.4	2.85	1.13	60.3
27-01-2014	1.18	0.45	61.9	3.38	1.27	62.4
28-01-2014	1.82	0.76	58.2	3.72	1.32	64.5
29-01-2014	1.70	0.65	61.9	3.76	1.47	60.9
30-01-2014	1.76	0.72	59.1	4.33	1.77	59.1
31-01-2014	1.63	0.69	57.6	4.24	1.77	58.2
03-02-2014	1.51	0.59	61.0	4.46	1.67	62.6
04-02-2014	1.55	0.56	63.9	3.95	1.57	60.3
05-02-2014	1.86	0.74	60.1	3.99	1.63	59.1
06-02-2014	1.64	0.61	62.7	4.56	1.76	61.4
07-02-2014	1.70	0.57	66.6	4.38	1.52	65.3

10-02-2014	1.58	0.67	57.6	3.80	2.01	47.1
11-02-2014	1.72	0.43	75.0	4.16	1.46	64.9
12-02-2014	2.71	1.17	56.8	4.72	1.98	58.1
13-02-2014	2.26	1.01	55.2	4.55	1.86	59.1
14-02-2014	2.03	0.54	73.4	4.48	1.01	77.5
Range (Min-Max)	**1.15-2.71**	**0.43-1.53**	**15.7-75**	**2.39-4.72**	**1.01-2.92**	**-9.4-77.5**
Mean ± S.D.	**1.70 ± 0.31**	**0.70 ±0.25**	**59.02 ±12.00**	**3.60 ±0.72**	**1.55 ±.38**	**55.24 ±15.61**

$^*AP_i$ = available phosphate at inlet; $^*AP_o$ = available phosphate at outlet; $^*TP_i$ = available phosphate at inlet; $^*TP_o$ = total phosphate at outlet

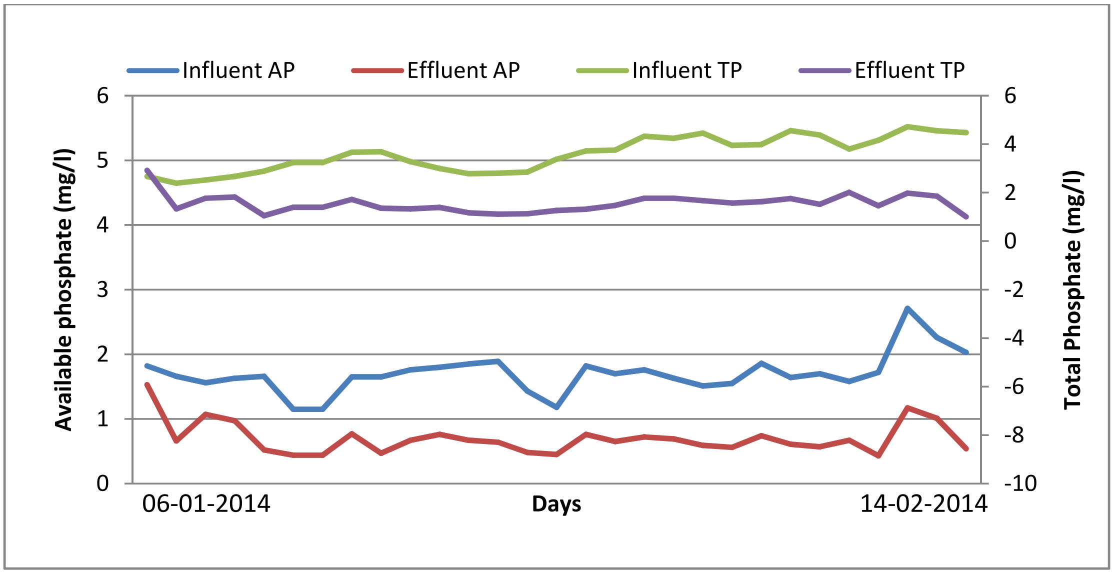

Figure 4.4: Available Phosphate and Total phosphate concentration (mg/l) in influent and effluent in winter

4.5.1.3 Spring Season

The study was initiated from mid February to end of March. During autumn and winter season it was observed that nitrogen is required by the plant as external input, or the plant could undergo stress. Therefore, during spring season, ammonium ions were amended to CW cell with the concentration of 50 mg/l in the form of ammonium sulphate ($(NH_4)_2SO_4$), and initial concentration of phosphate was raised to 10 mg/l. The other reason for addition of nitrogen in the form of ammonium ions was to make an indirect inference of nitrifying conditions in CW cell. The difference in inlet and outlet concentration of TKN comments upon nitrification of ammonium ions to nitrates and its uptake by *Brachiaria.* The initial concentration of TP in synthetic wastewater, was increased from 5mg/l to 10mg/l. Amendment of nitrogen resulted in improved removal of phosphate from wastewater having an average inlet concentration of 9.7 mg/l. The concentration of TP in influent varied from 6.76mg/l to 12.49mg/l during study. Available phosphate (AP) in synthetic waste water varied from 1.34mg/l to 5.6mg/l with an average of 3.65mg/l. The effluent obtained after an HRT of 24 hours had AP concentration varying from0.08mg/l to 1.25mg/l with an average of 0.70mg/l. Concentration of AP in influent and effluent represented removal efficiency varying from 65.02% to 94% with an average removal efficiency of 80.6% during the spring season as compared to 59% during winter season. The concentration of TP in effluent varied from 0.7 mg/l to 3.26mg/l with an average value of 2.04mg/l. Concentration of TP in influent and effluent represented removal efficiency varying from 63.01% to 93.31% resulting in an average removal efficiency of 78.45% as compared to 55.2% during winters. The improved removal efficiency may be attributed to abundance of nutrients in CW cell. The removal of phosphates from CW cell thus follows first order kinetics. Another reason for improved removal rate may be gradual increase in ambient temperature and average sunshine hours during the spring season. It rose from 16^{o}C to 25.5^{o}C during the period. An increase in temperature results in increased rate of evapotranspiration causing an increased updraft of water from roots to the leaves of *Brachiaria*. Evapotranspiration is, therefore, directly related to the removal efficiency of the plant. It was observed that the removal efficiency of *Brachiaria* for AP was slightly higher than that for TP. (Table 4.4, Figure 4.5)

The reason maybe ascribed to bio availability of AP compared to TP since TP is bound to different elemental entities and organic matter present in sediments and wastewater, its availability to the wetland vegetation is slightly less. The biotransformation of different chemical forms of phosphate results in the conversion of TP to AP. pH of the system, redox conditions and prescence of metals (Ca, Mg, Al, Fe etc) regulate the bio chemical conversion of phosphate. The results in the present study represents that conversion of non available/bound phosphate takes place to available phosphate, followed by the uptake by wetland vegetation preferably. The average removal rate was observed to be 175.69mg/m^{2}-day; ranging from 122.04mg/

m^2-day to 226.22mg/ m^2-day for TP for average inlet and outlet loading rate of 176.39mg/m^2-day and 0.69mg/m^2-day.

Table 4.4 Concentration of phosphate PO_4^{3-}-P (mg/l) and its removal efficiency (%) during spring season

Date (n=27)	Available Phosphate			Total Phosphate		
	Inlet (AP_i) (mg/l)	Outlet (AP_o) (mg/l)	Removal efficiency (%)	Inlet (TP_i) (mg/l)	Outlet (TP_o) (mg/l)	Removal efficiency (%)
17-02-2014	3.80	0.74	80.5	7.60	1.53	79.9
18-02-2014	3.44	0.95	72.3	6.76	2.5	63.0
19-02-2014	3.51	0.66	81.2	6.99	1.67	76.1
20-02-2014	3.67	1.23	66.6	7.90	2.7	65.8
21-02-2014	3.80	1.19	68.7	7.82	2.65	66.1
24-02-2014	3.57	1.25	65.0	8.18	2.51	69.3
25-02-2014	3.81	0.99	74.0	7.96	2.43	69.5
26-02-2014	2.65	0.44	83.3	9.38	2.67	71.5
27-02-2014	1.88	0.48	74.5	11.65	3.01	74.2
28-02-2014	3.96	0.72	81.8	12.49	2.56	79.5
03-03-2014	1.90	0.22	88.4	8.29	1.1	86.7
04-03-2014	1.34	0.08	94.0	10.46	0.7	93.3
05-03-2014	3.39	0.76	77.6	10.35	1.7	83.6
06-03-2014	4.86	0.66	86.5	10.91	2.7	75.3
07-03-2014	4.03	0.91	77.4	9.95	2.01	79.8
10-03-2014	3.33	0.55	83.5	11.03	1.9	82.8
11-03-2014	3.73	0.51	86.3	8.84	1.7	80.8
12-03-2014	4.63	0.55	88.1	10.79	1.12	89.6
13-03-2014	4.00	0.49	87.8	10.61	1.7	84.0
14-03-2014	4.17	0.67	83.9	9.33	1.6	82.9
18-03-2014	4.33	0.68	84.3	9.89	1.24	87.5
19-03-2014	3.75	0.79	79.0	9.79	1.87	80.9

20-03-2014	5.29	0.97	81.7	10.79	2.11	80.4
21-03-2014	2.30	0.71	69.1	10.28	2.43	76.4
24-03-2014	4.73	0.48	89.9	11.39	2.2	80.7
25-03-2014	5.60	0.48	91.4	11.28	1.4	87.6
26-03-2014	2.99	0.62	79.3	11.24	3.26	71.0
Range (Min-Max)	**1.34-5.6**	**0.08-1.25**	**65.02-94**	**6.76-12.49**	**0.7-3.26**	**63-93.3**
Mean ± S.D.	**3.65 ± 1.00**	**0.70 ±0.28**	**80.6 ±7.73**	**9.70 ±1.55**	**2.03 ±0.65**	**78.44 ±7.60**

[*]AP_i = available phosphate at inlet; [*]AP_o = available phosphate at outlet; [*]TP_i = available phosphate at inlet; [*]TP_o = total phosphate at outlet

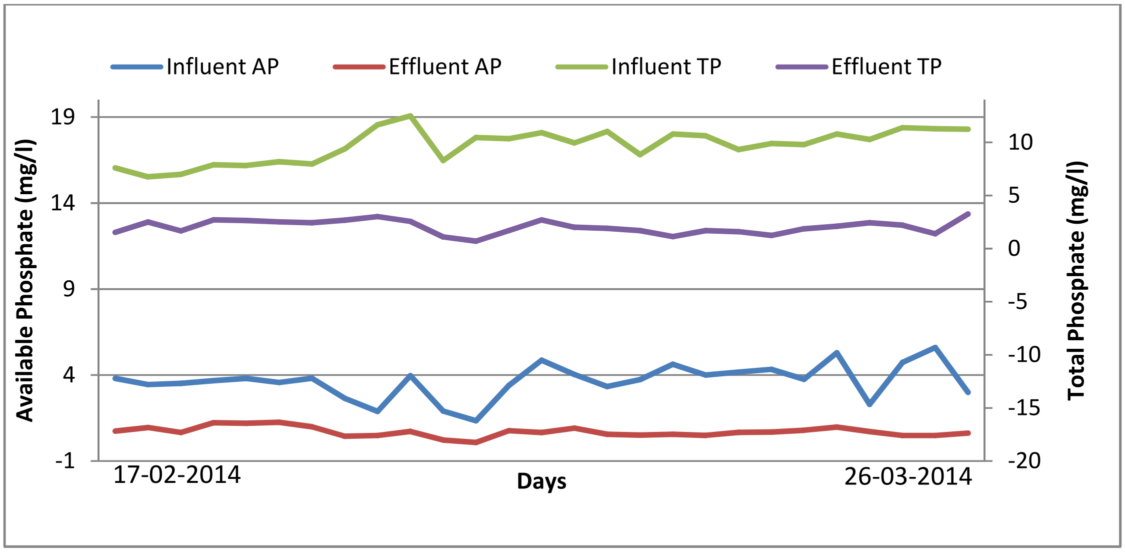

Figure 4.5: Available Phosphate and Total phosphate concentration (mg/l) in influent and effluent in spring season

4.5.1.4 Summer Season

Summer season was classified as the period from last week of March, 2014 to April, 2014 during the study. During this period, nitrogen and phosphorus were amended as ammonium (NH_4^+) and phosphate (PO_4^{3-}) ions at an inlet concentration of 100 mg/l and 20 mg/l, respectively, in influent. The average concentration of total phosphate in inlet was 21.20mg/l varying from 18.2 mg/l to 23.58 mg/l (Table 4.4; Fig. 4.6). Concentration of TP in influent and effluent represented removal efficiency varying from 71.88% to 94.89% with an average removal efficiency of 85.6%. Available phosphate (AP) in synthetic waste water varied from 5.26mg/l to 13.23mg/l with an average of 8.66mg/l. The effluent obtained after an HRT of 24 hours had AP concentration varying from0.34mg/l to 2.56mg/l with an average of 1.35mg/l. Concentration of AP in influent and effluent represented removal efficiency varying from 67.92% to 94.84% with an average removal efficiency of 83.9% during the summer season. Removal efficiency was significantly above the autumn and winter season. However, it was slightly below the removal efficiency of TP, which was a reversal of trend as seen in the earlier seasons.An improvement in removal efficiency with an increase in phosphate concentration represents first order kinetics in summer season too. TP is available to wetland vegetation only after its bio conversion to AP being regulated by pH of water and sediments, redox conditions in CW cell, availability of metals like Ca, Mg, Al, and Fe in sediments and wastewater.

The average removal rate was observed to be 384.4mg/m^2-day; ranging from 329.9mg/ m^2-day to 428.1mg/ m^2-day for TP for average inlet and outlet loading rate of 385.42mg/m^2-day and 1.02mg/m^2-day.

Table 4.5 Concentration of phosphate PO_4^{3-}-P (mg/l) and its removal efficiency (%) during summer season

Date (n=15)	**Available Phosphate**			**Total Phosphate**		
	Inlet (AP_i) (mg/l)	**Outlet (AP_o) (mg/l)**	**Removal efficiency (%)**	**Inlet (TP_i) (mg/l)**	**Outlet (TP_o) (mg/l)**	**Removal efficiency (%)**
27-03-2014	5.74	1.45	74.7	20.49	5.76	71.9
28-03-2014	13.23	1.48	88.8	21.76	3.1	85.8
31-03-2014	12.64	1.49	88.2	20.04	2.68	86.6
01-04-2014	7.34	0.97	86.8	23.14	3.76	83.7
02-04-2014	11.01	1.74	84.2	20.17	3.96	80.4
03-04-2014	10.19	1.56	84.7	19.85	3.83	80.7
04-04-2014	8.77	1.42	83.8	18.96	3.45	81.8
07-04-2014	8.93	0.46	94.8	20.57	2.38	88.4
12-04-2014	10.27	2.21	78.5	20.09	3.67	81.7
13-04-2014	7.98	2.56	67.9	18.20	2.87	84.2
15-04-2014	7.85	0.76	90.3	22.33	2.56	88.5
16-04-2014	6.63	0.64	90.3	23.29	1.62	93.0
17-04-2014	6.26	0.34	94.6	21.93	1.12	94.9
18-04-2014	7.82	1.66	78.8	23.57	1.9	91.9
19-04-2014	5.26	1.50	71.6	23.58	2.1	91.1
Mean ± S.D.	**8.66 ± 2.40**	**1.35 ±0.62**	**83.9 ±1.73**	**21.20 ±1.15**	**2.98 ±1.15**	**85.6 ±5.99**
Range (Min-Max)	**5.26-13.23**	**0.34-2.56**	**67.9-94.8**	**18.2-23.58**	**1.12-5.76**	**71.9-94.9**

$^{*}AP_i$ = available phosphate at inlet; $^{*}AP_o$ = available phosphate at outlet; $^{*}TP_i$ = available phosphate at inlet; $^{*}TP_o$ = total phosphate at outlet

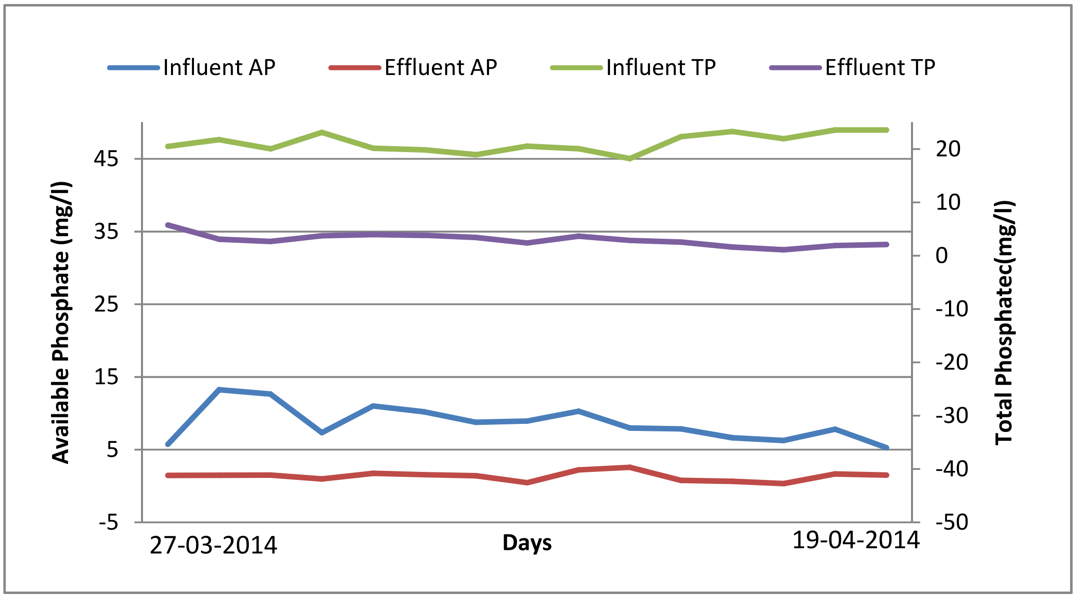

Figure 4.6 Available Phosphate and Total phosphate concentration (mg/l) in influent and effluent during the summer season

The removal of phosphate (AP and TP) by *Brachiaria* will chiefly be governed by the environmental conditions, and, therefore, the seasons have an effect over the removal behaviour of plants. Temperature is a significant parameter affecting removal rate for nutrients. Removal efficiency of phosphate was observed to be directly related to temperature since higher the evapotranspiration, more shall be the rate of uptake of water and nutrients from an aquatic system (Kadlec and Knight). Similar results were obtained in the present study with the only exception of a sudden drop in late autumn season (Fig. 4.6). The reason may be ascribed to nutrient stress over the plants. It is therefore, necessary to maintain the required supply of nutrients (CNP) in a wetland system. The removal efficiency of AP was placed slightly above the removal efficiency of TP and it increased through the seasons from autumn to spring but the trend reversed during summer when TP removal exceeded AP. However, the difference was not significant (Fig. 4.7).

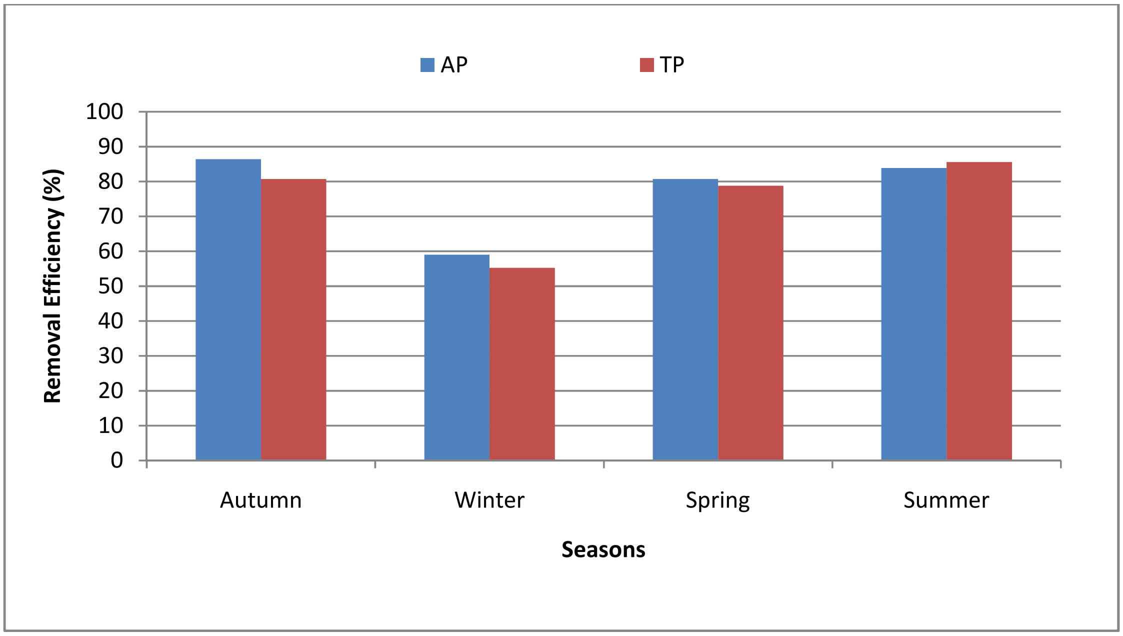

Figure 4.7 The variation in average removal efficiency of *Brachiaria mutica* in different seasons

4.6 Effect of initial concentration

The effect of initial influent concentration of phosphate on removal efficiency of *Brachiaria* was investigated to get an insight of adaptability, tolerance and response of the plant to varying chemical shocks of phosphate. The removal efficiency was studied at an initial concentration of 5 mgP/l, raised to 10 mgP/l amended with nitrogen (50 mg/l NH_4^+-N); and finally raised to 20 mgP/l supplemented with nitrogen (ammonium ions at 100 mg/l) with the monitoring of ferric (Fe^{3+}) and Ferrous (Fe^{2+}) ions in the treated wastewater. Phosphate was characterised as AP and TP during the study. Based on the concentration of AP and TP in effluent, removal efficiency (%) and removal rate (mg/m^2-day) were calculated. The nitrifying conditions in CW cell were monitored on the basis of TKN concentration in influent and effluent. The redox conditions of the CW cell were also monitored on the basis of ferrous and ferric ions concentration in effluent at very high nutrient loading. The results so obtained at varying phosphate loading rates are discussed below.

4.6.1 Available Phosphates (AP) and Total Phosphates (TP)

Available and total phosphate from the synthetically prepared waste water for inlet and treated water after the retention period of 24 hours as outlet from the constructed wetland cell was analysed. Available and total phosphate were analysed for both inlet and outlet. Concentration (mg/l) and removal efficiency (%) of available and total phosphate by the *Brachiaria* was analysed during the study and the loading rate (mgP/m^2-day) was also determined for both inlet and outlet.

4.6.1.1 Removal at 5 mg/l PO_4^{3-}-P Inlet Concentration

The initial loading of phosphate was kept at a moderate value of 5 mg/l. The average inlet concentration was 3.75 mg/l as total phosphate with a deviation of ±1.03 mg/l. The value of average AP in inlet was 1.57 mg/l with a deviation of ±0.66 mg/l against the average concentration of 0.32 mg/l of AP in effluent (varying from0.1mg/l to 0.54mg/l). Average removal efficiency of 82.09% was observed for AP as compared to 83.10 % for TP (Table 4.6). The average removal rate of TP was 61.6 mgP/m^2-day as against the influent loading rate of 61.97mgP/m^2-day. The removal rate of TP was observed to be significantly high at the present loading rate. During the study, a sharp decrease in removal efficiency and rate was observed after a period of one month. The decrease may be attributed to the stressed growth of *Brachiaria* in later stage owing to chemical stress on account of deficiency of nitrogen. Since phosphate was added as the only nutrient in synthetic wastewater, nitrogen deficient conditions were created after the period of around 30 days. Initial requirement might be met by the nitrates present in the sediments. The other reason might be the decrease in average ambient temperature from 25.5^{o}C (October) to about 19^{o}C (November) resulting in lower metabolic rates of *Brachiaria* at lower temperature on account of onset of transformation of season from autumn to winter. At the same time average sunshine hours reduced resulting in reduced productivity. It had resulted in chlorosis of leaves and onset of drying of stems. During this period (December 07, 2013 to January 06, 2014), efforts were made to revive the health of *Brachiaria*, and analysis of phosphate and other characteristics of wastewater remained suspended. In order to revive the plant health, temporary agro-net was removed to facilitate enhanced exposure to sunlight; phosphate was amended with Di-ammonium phosphate (DAP) and urea at regular interval of ten days; and the hydraulic loading was reduced. New leaves and lateral branches generated after a period of around 15 days and the plants had stabilised in a period of about 30 days. Following it the regular analysis as started initially was initiated. Later, it was observed that removal efficiency of AP and TP improved significantly and stabilised at ≈60% for AP and TP (Table 4.6). The removal rate too improved gradually and stabilised at ≈70 mgP/m^2-day in the later stage. The removal efficiency of TP exceeded the removal of AP in later stage owing to removal of TP by sediments. The removal rate increased gradually with slight variations (±10.1 mgP/m^2-day) during the study (Fig. 4.8).

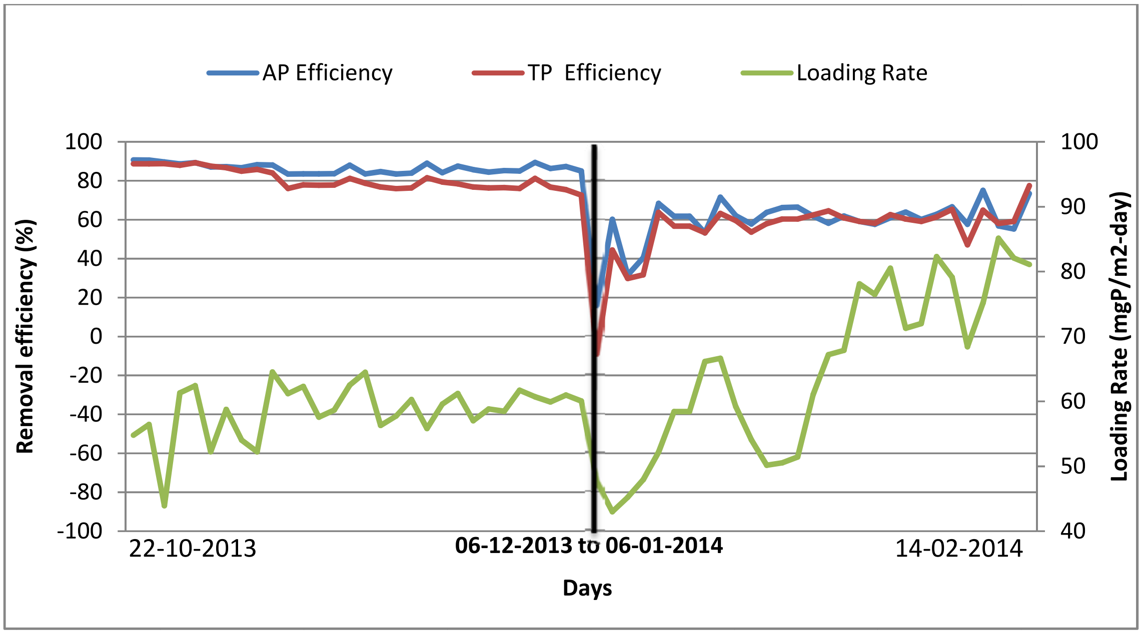

Figure 4.8: AP and TP removal efficiency (%) and loading rate (mgP/m^2-day) for the influent phosphate concentration of 5 mg/l

Table4.6: Removal efficiency (%) and removal rate (mg/m^2-day) of PO_4^{3-}–P by *B. mutica* at an influent concentration of 5mgP/l

Date (n=59)	Inlet					Outlet					Removal Efficiency in %		Loading Rate in mg/m^2-day		Removal Rate (mg/m^2-day)
	pH	TDS in ppm	Conductivity (µS/cm)	AP (mg/l)	TP (mg/l)	pH	TDS in ppm	Conductivity (µS/cm)	AP (mg/l)	TP (mg/l)	AP	TP	Inlet	Outlet	
22-10-2013	8	361	722	1.1	3.02	8.34	765	1530	0.10	0.34	90.7	88.7	54.91	0.12	54.79
23-10-2013	8	360	720	1.17	3.11	8.1	815	1630	0.11	0.35	90.6	88.7	56.55	0.12	56.43
24-10-2013	8	357.5	715	1.15	2.42	8.04	819.5	1639	0.12	0.27	89.6	88.8	44.00	0.09	43.91
25-10-2013	8	375	750	1.14	3.38	7.6	907	1814	0.13	0.41	88.6	87.9	61.45	0.14	61.31
28-10-2013	7.9	367.5	735	1.13	3.44	7.3	983.5	1967	0.12	0.37	89.3	89.2	62.55	0.13	62.42
29-10-2013	8	370.5	741	1.02	2.88	7.33	1016	2032	0.13	0.36	87.1	87.5	52.36	0.12	52.24
30-10-2013	7.9	370.5	741	1.07	3.24	7.13	1054	2108	0.14	0.43	87.2	86.7	58.91	0.15	58.76
31-10-2013	7.9	370	740	1.1	2.98	7.2	1103.5	2207	0.15	0.45	86.7	84.9	54.18	0.15	54.03
01-11-2013	7.8	367	734	1.13	2.88	7.1	1152	2304	0.13	0.41	88.2	85.8	52.36	0.14	52.22

04-11-2013	7.6	369	738	1.15	3.56	7.03	1168.5	2337	0.14	0.57	88.1	84	64.73	0.19	64.53
05-11-2013	7.7	366.5	733	1.02	3.38	7.04	1190	2380	0.17	0.81	83.4	76	61.45	0.28	61.18
06-11-2013	7.8	366.5	733	1.13	3.44	7.03	1192	2384	0.19	0.76	83.5	77.9	62.55	0.26	62.29
07-11-2013	7.9	367	734	1.1	3.18	7.02	1192	2384	0.18	0.71	83.5	77.7	57.82	0.24	57.58
08-11-2013	8	367.5	735	1.07	3.24	7.08	1215	2430	0.18	0.72	83.6	77.8	58.91	0.25	58.66
11-11-2013	7.9	368	736	1	3.45	6.95	1188.5	2377	0.12	0.65	88	81.2	62.73	0.22	62.51
12-11-2013	7.76	369	738	1.13	3.56	6.95	1190	2380	0.19	0.76	83.5	78.7	64.73	0.26	64.47
13-11-2013	7.82	370	740	1.19	3.11	6.94	1192	2384	0.18	0.72	84.7	76.8	56.55	0.25	56.30
14-11-2013	7.83	370	740	1.12	3.19	6.92	1215	2430	0.19	0.77	83.4	75.9	58.00	0.26	57.74
15-11-2013	7.85	370.5	741	1.09	3.33	7.19	1252.5	2505	0.18	0.79	83.9	76.3	60.55	0.27	60.28
21-11-2013	7.85	349	698	1.15	3.08	7.2	1255.5	2511	0.13	0.57	89	81.5	56.00	0.19	55.81
22-11-2013	7.92	360	720	1.11	3.29	6.9	1260.5	2521	0.18	0.68	84.1	79.3	59.82	0.23	59.59
25-11-2013	7.96	357.5	715	1.02	3.38	6.85	989	1978	0.13	0.73	87.5	78.4	61.45	0.25	61.21
26-11-2013	7.93	361.5	723	1.09	3.15	6.93	1153	2306	0.16	0.73	85.7	76.8	57.27	0.25	57.02
27-11-2013	7.89	365	730	1	3.25	7.1	1174	2348	0.16	0.77	84.4	76.3	59.09	0.26	58.83
28-11-2013	7.86	360	720	1.2	3.23	7.28	1277.5	2555	0.18	0.76	85.2	76.5	58.73	0.26	58.47
29-11-2013	7.93	371.5	743	1.21	3.41	6.94	1269.5	2539	0.18	0.82	85	76	62.00	0.28	61.72
02-12-2013	7.89	375	750	1.17	3.35	6.87	1270	2540	0.12	0.63	89.4	81.2	60.91	0.21	60.69
03-12-2013	7.91	376	752	1.06	3.31	6.97	1283	2566	0.14	0.77	86.3	76.7	60.18	0.26	59.92
05-12-2013	7.93	378	756	1.1	3.37	6.88	1272.5	2545	0.14	0.83	87.3	75.4	61.27	0.28	60.99

06-12-2013	7.9	377.5	755	1.31	3.32	6.81	1168.5	2337	0.20	0.91	85	72.6	60.36	0.31	60.05
06-01-2014	6.69	279	554	1.82	2.67	6.62	1279	2558	1.53	2.92	15.7	-9.4	48.55	1.00	47.55
07-01-2014	6.7	280	569	1.66	2.39	6.62	898	2070	0.66	1.33	60.2	44.4	43.45	0.45	43.00
08-01-2014	6.82	285	580	1.56	2.52	6.71	935	2134	1.07	1.77	31.5	29.8	45.82	0.60	45.21
09-01-2014	6.84	289	585	1.63	2.67	6.65	887	2096	0.97	1.82	40.4	31.7	48.55	0.62	47.93
10-01-2014	6.93	284	578	1.66	2.89	6.69	862	2102	0.52	1.05	68.4	63.7	52.55	0.36	52.19
13-01-2014	7.11	278	566	1.15	3.24	6.65	863	2182	0.44	1.4	61.8	56.7	58.91	0.48	58.43
15-01-2014	7.17	278	566	1.15	3.24	6.67	882	2026	0.44	1.4	61.8	56.7	58.91	0.48	58.43
16-01-2014	7.38	346	705	1.65	3.67	6.77	1156	2514	0.77	1.72	53.2	53.2	66.73	0.59	66.14
17-01-2014	7.4	351	709	1.65	3.69	6.64	1159	2537	0.47	1.36	71.5	63.2	67.09	0.46	66.63
20-01-2014	7.25	330	701	1.76	3.28	6.76	950	1332	0.67	1.33	62	59.5	59.64	0.45	59.18
21-01-2014	7.1	329	690	1.8	3	6.81	915	1757	0.76	1.39	57.8	53.6	54.55	0.47	54.07
22-01-2014	6.91	315	696	1.85	2.78	6.75	672	1664	0.67	1.17	63.7	57.9	50.55	0.40	50.15
23-01-2014	6.93	282	574	1.89	2.8	6.63	853	1785	0.64	1.11	66.2	60.3	50.91	0.38	50.53
24-01-2014	7.23	281	573	1.43	2.85	6.77	870	1880	0.48	1.13	66.4	60.3	51.82	0.39	51.43
27-01-2014	6.91	282	574	1.18	3.38	6.62	917	1871	0.45	1.27	61.9	62.4	61.45	0.43	61.02
28-01-2014	6.89	291	569	1.82	3.72	6.71	772	1153	0.76	1.32	58.2	64.5	67.64	0.45	67.19
29-01-2014	6.91	284	580	1.7	3.76	6.7	778	1577	0.65	1.47	61.9	60.9	68.36	0.50	67.86
30-01-2014	7.35	290	591	1.76	4.33	6.71	478	1632	0.72	1.77	59.1	59.1	78.73	0.60	78.12
31-01-2014	7.4	296	594	1.63	4.24	6.85	511	1559	0.69	1.77	57.6	58.2	77.09	0.60	76.49

03-02-2014	7.6	290	582	1.51	4.46	6.9	561	1622	0.59	1.67	61	62.6	81.09	0.57	80.52
04-02-2014	7.3	298	593	1.55	3.95	6.91	539	1422	0.56	1.57	63.9	60.3	71.82	0.54	71.28
05-02-2014	7.1	279	578	1.86	3.99	6.96	650	1463	0.74	1.63	60.1	59.1	72.55	0.56	71.99
06-02-2014	7.2	293	583	1.64	4.56	6.89	768	1516	0.61	1.76	62.7	61.4	82.91	0.60	82.31
07-02-2014	7.1	299	597	1.7	4.38	6.9	750	1505	0.57	1.52	66.6	65.3	79.64	0.52	79.12
10-02-2014	7	280	585	1.58	3.8	7	880	1524	0.67	2.01	57.6	47.1	69.09	0.69	68.41
11-02-2014	7.1	287	573	1.72	4.16	7	889	1511	0.43	1.46	75	64.9	75.64	0.50	75.14
12-02-2014	7	292	594	2.71	4.72	7	913	1521	1.17	1.98	56.8	58.1	85.82	0.68	85.14
13-02-2014	7.11	293	598	2.26	4.55	7	968	1433	1.01	1.86	55.2	59.1	82.73	0.63	82.09
14-02-2014	7	278	586	2.03	4.48	7	833	1562	0.54	1.01	73.4	77.5	81.45	0.34	81.11
Range (Max-Min)	**6.69-8.0**	**278-378**	**554-756**	**1.00-2.71**	**2.39-4.72**	**6.62-8.34**	**478-1283**	**1153-2566**	**0.1-1.53**	**0.27-2.92**	**15.7-90.7**	**-9.4-89.2**	**43.45-85.82**	**0.09-1.00**	**43.00-85.14**
Mean ± S.D.	**7.49 ±0.44**	**331.41 ±39.52**	**668.66 ±75.49**	**1.40 ±0.37**	**3.41 ±0.55**	**6.98 ±0.34**	**989.36 ±221.5**	**2025.0 ±413.8**	**0.42 ±0.33**	**1.08 ±0.55**	**72.95 ±16.21**	**68.19 ±17.2**	**61.97 ±10.08**	**0.37 ±0.19**	**61.6 ±10.01**

4.6.1.2 Removal at 10 mg/l PO_4^{3-}-P Inlet Concentration

During this study TP concentration in influent was increased to 10 mg/l representing high strength wastewater with respect to phosphate. Nitrogen was also amended with the concentration of 50 mg/l as ammonium ions to get an indirect inference of nitrifying conditions. Nitrifying conditions comment upon/ represent oxidising environment in the CW cell, which in turn comments upon the binding of phosphate with the metal species present in sediments. Addition of ammonium ions also helped nitrifying bacteria stay functional/ active in the aquatic environment. The average concentration of TP in influent was found to be 9.7 mg/l with a deviation of ±1.6 mg/l as against an average concentration of 2.0 mg/l in effluent. It resulted in an average removal efficiency of 78.45 % for TP. The removal efficiency of AP was almost of the same order, about 80.6%, with the average influent and effluent concentration of 3.56 mg/l and 0.70 mg/l, respectively (Table 4.7; Fig. 4.9). The average removal rate of phosphate was observed to be 175.7 mg/m^2-day as against the initial phosphate loading of 176.4 mg/m^2-day. The phosphate removal rate of *Brachiaria* was therefore, observed to be substantially high even for high strength wastewater with respect to phosphate.

The possibility of phosphate being removed by sediments was ruled out based on the concentration of TKN in influent and effluent. An average influent concentration of 46.5 mg/l NH_4^+-N was maintained during the period. The average concentration of TKN in effluent was observed to be 11.26 mg/l resulting in average nitrogen removal efficiency of 76% (Table 4.8). This indicates that most of the ammonium ions are converted to nitrates which are subsequently taken up by the plants for growth and metabolism. The removal rate of TKN was observed to be 841.67 mg/m^2-day as against the influent loading of 845.5 mg/m^2-day. Since there is significant decrease in NH_4^+/ TKN, oxidising conditions prevailed in the CW cell during the period. Since binding of phosphate with metallic species does not take place under anaerobic conditions, and in neutral pH range; most of the removal of phosphate in the present study is by *Brachiaria* (Brix *et al.*,2001; Vymazal, 2004).

Table 4.7: Removal efficiency (%) and removal rate (mg/m^2-day) of PO_4^{3-}–P by *B. mutica* at an influent concentration of 10mgP/l

Date (n=27)	**Inlet**					**Outlet**					**Removal Efficiency (%)**		**Loading Rate (mg/m^2-day)**		**Removal Rate (mg/m^2-day)**
	pH	**TDS in ppm**	**Conductivity (µS/cm)**	**AP (mg/l)**	**TP (mg/l)**	**pH**	**TDS in ppm**	**Conductivity (µS/cm)**	**AP (mg/l)**	**TP (mg/l)**	**AP**	**TP**	**Inlet**	**Outlet**	
17-02-2014	7	428	872	3.80	7.60	7	630	1489	0.74	1.53	80.5	79.9	138.13	0.52	137.60
18-02-2014	7	444	906	3.44	6.76	6.75	724	1476	0.95	2.5	72.3	63.0	122.89	0.85	122.04
19-02-2014	7.51	431	879	3.51	6.99	6.73	736	1504	0.66	1.67	81.2	76.1	127.05	0.57	126.48
20-02-2014	7.58	391	813	3.67	7.90	6.72	808	1543	1.23	2.7	66.6	65.8	143.67	0.92	142.75

21-02-2014	7.43	452	922	3.80	7.82	6.69	744	1517	1.19	2.65	68.7	66.1	142.11	0.90	141.20
24-02-2014	7.49	365	744	3.57	8.18	6.64	851	1737	1.25	2.51	65.0	69.3	148.69	0.86	147.83
25-02-2014	7.55	363	739	3.81	7.96	6.79	825	1683	0.99	2.43	74.0	69.5	144.70	0.83	143.88
26-02-2014	7.6	366	784	2.65	9.38	6.23	821	1655	0.44	2.67	83.3	71.5	170.55	0.91	169.64
27-02-2014	7.6	370	799	1.88	11.65	6.75	896	1719	0.48	3.01	74.5	74.2	211.82	1.03	210.79
28-02-2014	7.8	396	841	3.96	12.49	6.89	891	1789	0.72	2.56	81.8	79.5	227.09	0.87	226.22
03-03-2014	7.9	404	859	1.90	8.29	6.99	817	1777	0.22	1.1	88.4	86.7	150.73	0.38	150.35
04-03-2014	8	445	897	1.34	10.46	7.13	823	1767	0.08	0.7	94.0	93.3	190.18	0.24	189.94
05-03-2014	8	415	899	3.39	10.35	7.19	835	1801	0.76	1.7	77.6	83.6	188.18	0.58	187.60
06-03-2014	8.1	479	910	4.86	10.91	7.29	897	1810	0.66	2.7	86.5	75.3	198.36	0.92	197.44
07-03-2014	8.1	459	914	4.03	9.95	7.1	852	1829	0.91	2.01	77.4	79.8	180.91	0.69	180.22
10-03-2014	8	471	961	3.33	11.03	7.3	879	1871	0.55	1.9	83.5	82.8	200.55	0.65	199.90
11-03-2014	8.2	452	921	3.73	8.84	7.4	835	1858	0.51	1.7	86.3	80.8	160.73	0.58	160.15
12-03-2014	8.1	465	951	4.63	10.79	7.4	851	1937	0.55	1.12	88.1	89.6	196.18	0.38	195.80

13-03-2014	8.1	474	965	4.00	10.61	7.4	902	2141	0.49	1.7	87.8	84.0	192.91	0.58	192.33
14-03-2014	8.2	538	1098	4.17	9.33	7.4	944	2216	0.67	1.6	83.9	82.9	169.64	0.55	169.09
18-03-2014	8.2	513	1045	4.33	9.89	7.5	951	2143	0.68	1.24	84.3	87.5	179.82	0.42	179.40
19-03-2014	8.3	505	1020	3.75	9.79	7.5	950	2315	0.79	1.87	79.0	80.9	178.00	0.64	177.36
20-03-2014	8.3	500	1017	5.29	10.79	7.4	978	2337	0.97	2.11	81.7	80.4	196.18	0.72	195.46
21-03-2014	8.2	499	1019	2.30	10.28	7.8	979	2431	0.71	2.43	69.1	76.4	186.91	0.83	186.08
24-03-2014	7.9	499	1020	4.73	11.39	7.8	979	2439	0.48	2.2	89.9	80.7	207.09	0.75	206.34
25-03-2014	7.9	500	1011	5.60	11.28	7.1	976	2495	0.48	1.4	91.4	87.6	205.09	0.48	204.61
26-03-2014	8	496	1387	2.99	11.24	7.3	980	2510	0.62	3.26	79.3	71.0	204.36	1.11	203.25
Range (min-max)	**7-8.3**	**363-583**	**739 - 1387**	**1.34-5.6**	**6.76 - 12.49**	**6.23-7.8**	**630-980**	**1476-2510**	**0.08-1.25**	**0.7-3.26**	**65 - 94**	**63-93.3**	**122.9-227.1**	**0.24-1.11**	**122.04-226.22**
Mean ±S.D.	**7.85 ±0.36**	**448.9 ±51.02**	**933.07± 129.6**	**3.65 ±1.01**	**9.70 ±1.55**	**7.12± 0.38**	**864.96 ±89.66**	**1918.1 ±333.3**	**0.70 ±0.28**	**2.04 ±0.64**	**80.60 ±7.72**	**78.45± 7.74**	**176.4 ±28.2**	**0.69 ±0.22**	**175.69±28.23**

Table 4.8: Total Kjeldahl's nitrogen concentration (mg/l), its removal efficiency (%) and removal rate (mg/m^2-day) at influent concentration of 50mgN/l

Date (n=26)	**TKN(mg/l)**		**Removal Efficiency (%)**	**Loading rate(mg/m^2-day)**		**Removal Rate (mg/m^2-day)**
	Inlet (mg/l)	**Outlet(mg/l)**		**Inlet**	**Outlet**	
18-02-2014	33.6	12.6	62.5	610.9	4.3	606.6
19-02-2014	48	12.13	74.73	872.7	4.1	868.6
20-02-2014	41.53	16.23	60.92	755.1	5.5	749.6
21-02-2014	51.9	14.9	71.29	943.6	5.1	938.6
24-02-2014	49	12.13	75.24	890.9	4.1	886.8
25-02-2014	39.67	11.67	70.58	721.3	4.0	717.3
26-02-2014	48.4	14	71.07	880.0	4.8	875.2
27-02-2014	48.4	8.87	81.67	880.0	3.0	877.0
28-02-2014	42.2	11.2	73.46	767.3	3.8	763.5
3-03-2014	40.6	9.8	75.86	738.2	3.3	734.8
4-03-2014	46	11.2	75.65	836.4	3.8	832.5
5-03-2014	42	14	66.67	763.6	4.8	758.9

6-03-2014	49.87	7.7	84.56	906.7	2.6	904.1
7-03-2014	49	17.2	64.9	890.9	5.9	885.0
10-03-2014	49	7	85.71	890.9	2.4	888.5
11-03-2014	49	8.4	82.86	890.9	2.9	888.0
12-03-2014	49.7	11.2	77.46	903.6	3.8	899.8
13-03-2014	42	11.2	73.33	763.6	3.8	759.8
14-03-2014	46.2	11.2	75.76	840.0	3.8	836.2
18-03-2014	49	7	85.71	890.9	2.4	888.5
19-03-2014	49	11.2	77.14	890.9	3.8	887.1
20-03-2014	49	9.8	80	890.9	3.3	887.6
21-03-2014	49	9.8	80	890.9	3.3	887.6
24-03-2014	49	9.8	80	890.9	3.3	887.6
25-03-2014	49	12.6	77.14	890.9	4.3	886.6
26-03-2014	49	9.8	80	890.9	3.3	887.6
Range (Min-Max)	**33.6-51.9**	**7-17.2**	**60.92-85.71**	**610.91-943.64**	**2.39-5.86**	**606.61-938.56**
Mean ± S.D.	**46.50±4.29**	**11.255±2.59**	**75.55±6.68**	**845.5±78.07**	**3.84±0.88**	**841.67±78.27**

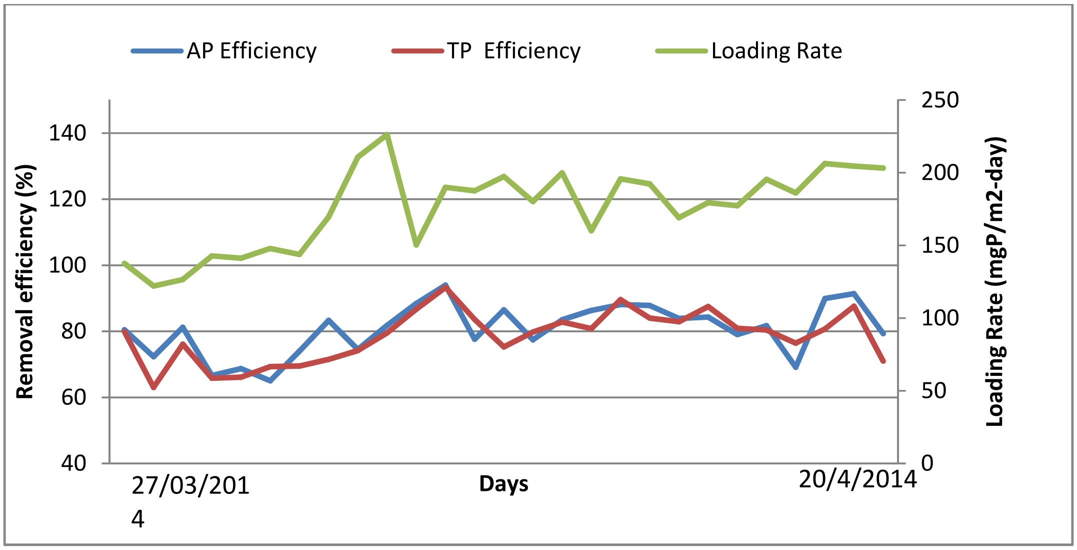

Figure 4.9 AP and TP removal efficiency (%) and loading rate (mgP/m^2-day) for the influent phosphate concentration of 10 mg/l

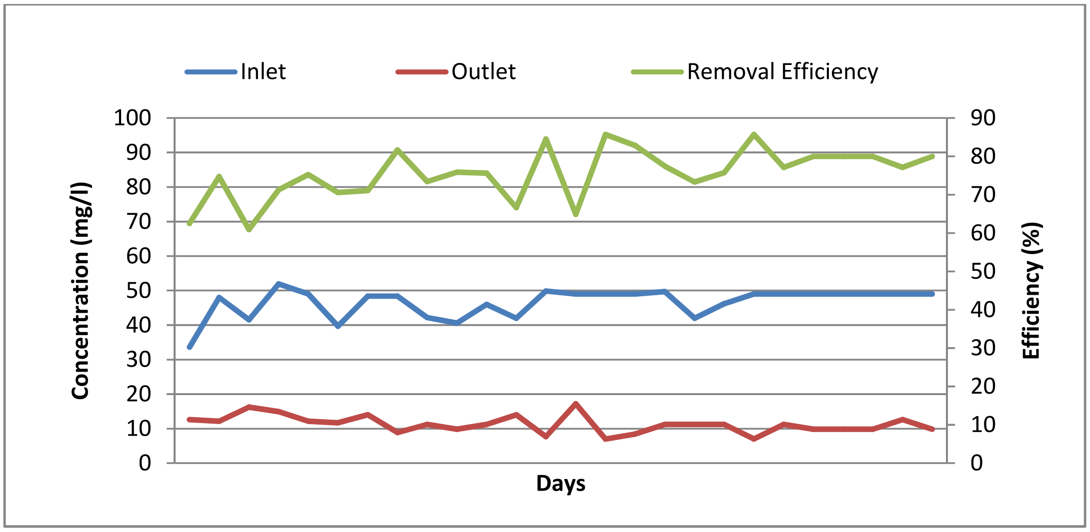

Figure 4.10: TKN concentrations (mg/l) and its removal efficiency (%) for influent concentration of 50 mg/l

4.6.1.3 Removal at 20 mg/l PO_4^{3-}-P Inlet Concentration

The TP concentration in influent was increased to 20 mg/l representing high strength wastewater with respect to phosphate. NH_4^+-N was also amended to the CW cell with the concentration of 100 mg/l so as to get an indirect inference of nitrifying conditions at the bed level. Nitrifying conditions comment upon/ represent oxidising environment in the CW cell, which in turn comments upon the binding of phosphate with the metal species present in sediments. Addition of ammonium ions also helped nitrifying bacteria to stay functional/ active in the aquatic environment. The average concentration of TP in influent was found to be 21.20 mg/l with a deviation of ±1.73 mg/l as against an average concentration of 2.98mg/l in effluent with slight deviation of ±1.15 mg/l. It resulted in an average removal efficiency of 85.6% for TP. The removal efficiency of AP was 83.9% which is almost of the same order with the average influent and effluent concentration of 8.66 mg/l and 1.35 mg/l, respectively (Table 4.9; Fig. 4.11). The average removal rate of phosphate was observed to be 384.40 mg/m^2-day as against the average influent phosphate loading of 385.42 mg/m^2-day. The phosphate removal rate of *Brachiaria mutica* was, therefore, observed to be substantially high even for high strength wastewater with respect to phosphate.

The possibility of phosphate being removed by sediments can ruled out based on the concentration of TKN in influent and effluent. An average influent concentration of 99.23 mg/l NH_4^+-N was maintained during the period. The average concentration of TKN in effluent was observed to be 15.3 mg/l resulting in average nitrogen removal efficiency of 84.6% with deviation of ±3.47% (Table 4.10). This indicates that most of the ammonium ions are converted to nitrates by its nitrification which is subsequently taken up by the plants for growth and metabolism. The removal rate of TKN was observed to be 1798.9 mg/m^2-day as against the influent loading of 1804 mg/m^2-day. Since there is significant decrease in NH_4^+/ TKN, oxidising conditions prevailed in the CW cell during the period. Since binding of phosphate with metallic species does not take place under aerobic conditions, and in neutral pH range; most of the removal of phosphate in the present study is by *Brachiaria* (Brix *et al.*,2001; Vymazal, 2004).

Table 4.9: Removal efficiency (%) and removal rate (mg/m^2-day) of PO_4^{3-}–P by *B. mutica* at an influent concentration of 20mgP/l

Date	Inlet					Outlet					Removal Efficiency (%)		Loading Rate (mg/m^2-day)		
	pH	TDS (ppm)	Conductivity (µS/cm)	AP (mg/l)	TP (mg/l)	pH	TDS (ppm)	Conductivity (µS/cm)	AP (mg/l)	TP (mg/l)	AP	TP	Inlet	Outlet	Removal Rate (mg/m^2-day)
27-03-2014	7.8	680	1395	5.74	20.49	7	981	2611	1.45	5.76	74.7	71.9	372.47	1.96	370.51
28-03-2014	7.8	685	1425	13.23	21.76	7.1	1050	2701	1.48	3.1	88.8	85.8	395.56	1.06	394.51
31-03-2014	7.8	699	1429	12.64	20.04	7.1	1249	2723	1.49	2.68	88.2	86.6	364.36	0.91	363.45
01-04-2014	7.8	658	1383	7.34	23.14	7.1	1150	2805	0.97	3.76	86.8	83.7	420.69	1.28	419.41
02-04-2014	7.8	681	1420	11.01	20.17	7	1115	2735	1.74	3.96	84.2	80.4	366.76	1.35	365.41
03-04-2014	7.9	696	1421	10.19	19.85	7.1	1269	2825	1.56	3.83	84.7	80.7	360.98	1.31	359.68
04-04-2014	7.9	680	1389	8.77	18.96	7.2	1339	2765	1.42	3.45	83.8	81.8	344.76	1.18	343.59
07-04-2014	8	691	1399	8.93	20.57	7.4	1250	2727	0.46	2.38	94.8	88.4	374.07	0.81	373.26
12-04-2014	7.9	692	1402	10.27	20.09	7.1	1939	2815	2.21	3.67	78.5	81.7	365.35	1.25	364.09
13-04-2014	8	687	1388	7.98	18.20	7.2	1780	2885	2.56	2.87	67.9	84.2	330.84	0.98	329.86

15-04-2014	8.2	697	1414	7.85	22.33	7.4	2189	2779	0.76	2.56	90.3	88.5	406.00	0.87	405.13
16-04-2014	7.9	694	1441	6.63	23.29	7.1	2212	2987	0.64	1.62	90.3	93.0	423.45	0.55	422.90
17-04-2014	7.9	693	1401	6.26	21.93	7.1	2369	3032	0.34	1.12	94.6	94.9	398.73	0.38	398.35
18-04-2014	7.9	699	1403	7.82	23.57	7.1	2355	3432	1.66	1.9	78.8	91.9	428.51	0.65	427.86
19-04-2014	7.9	698	1396	5.26	23.58	7	2386	3347	1.50	2.1	71.6	91.1	428.76	0.72	428.05
20-04-2014	7.9	691	1419			7	2480	3960							
Range (Min-Max)	**7.8-8.2**	**658-699**	**1383-1441**	**5.26-13.23**	**18.2-23.58**	**7-7.4**	**981-2480**	**2611-3960**	**0.34-2.56**	**1.12-5.76**	**67.9-94.8**	**71.9-94.9**	**330.84-428.76**	**0.38-1.96**	**329.86-428.05**
Mean ±S.D.	**7.90 ±0.10**	**688.8 ±10.5**	**1407.8 ±16.69**	**8.66 ±2.40**	**21.20 ±1.73**	**7.13 ±0.1**	**1694.6 ±567.2**	**2945.56 ±352.59**	**1.35 ±0.62**	**2.98 ±1.15**	**83.87 ±8.08**	**85.64 ±5.99**	**385.42 ±31.45**	**1.02 ±0.39**	**384.40 ±31.63**

Table 4.10: TKN concentration (mg/l), its removal efficiency (%) and removal rate (mg/m^2-day) at influent concentration of 100mgN/l

Date (n=16)	**TKN**		**Removal Efficiency (%)**	**loading rate(mg/m^2-day)**		**Removal Rate (mg/m^2-day)**
	Inlet (mg/l)	**Outlet (mg/l)**		**Inlet**	**Outlet**	
27-03-2014	99.4	9.8	90.1	1807.3	3.3	1803.9
28-03-2014	99.4	9.8	90.1	1807.3	3.3	1803.9
31-03-2014	99.4	14	85.9	1807.3	4.8	1802.5
1-04-2014	99.4	15.4	84.5	1807.3	5.3	1802.0
2-04-2014	99.4	18.2	81.7	1807.3	6.2	1801.1
3-04-2014	99.4	21	78.9	1807.3	7.2	1800.1
4-04-2014	99.4	21	78.9	1807.3	7.2	1800.1
7-04-2014	98	15.4	84.3	1781.8	5.3	1776.6
12-04-2014	99.4	21	78.9	1807.3	7.2	1800.1
13-04-2014	99.4	14	85.9	1807.3	4.8	1802.5
15-04-2014	99.4	14	85.9	1807.3	4.8	1802.5
16-04-2014	99.4	15.4	84.5	1807.3	5.3	1802.0

17-04-2014	99.4	14	85.9	1807.3	4.8	1802.5
18-04-2014	98	14	85.7	1781.8	4.8	1777.0
19-04-2014	99.4	14	85.9	1807.3	4.8	1802.5
20-04-2014	99.4	14	85.9	1807.3	4.8	1802.5
Range (Min-Max)	**98.0-99.4**	**9.8-21**	**78.87-90.14**	**1781.8-1807.3**	**3.3-7.2**	**1776.6-1803.9**
Mean ± S.D.	**99.2 ±0.48**	**15.3 ±3.4**	**84.6 ±3.5**	**1804.1 ±8.7**	**5.2 ±1.2**	**1798.9 ±8.7**

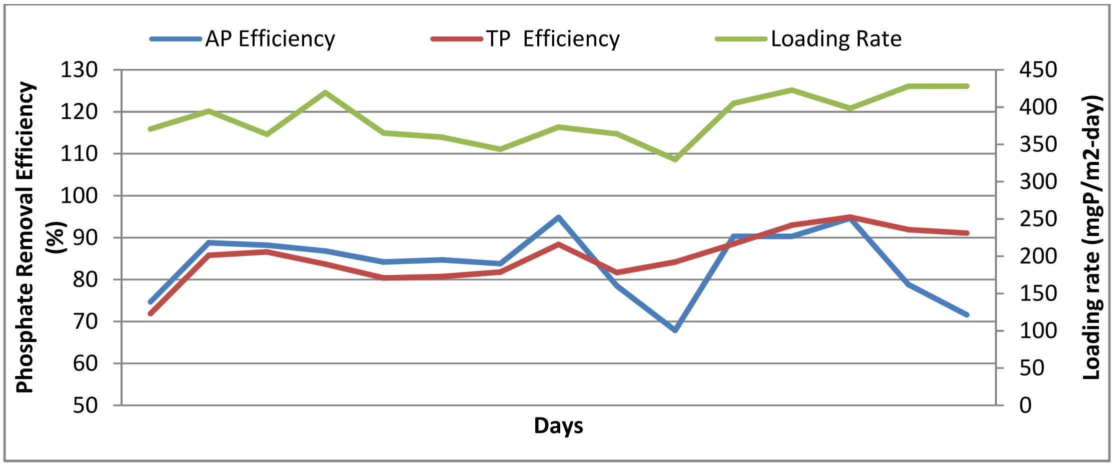

Figure 4.11 AP and TP removal efficiency (%) and loading rate (mgP/m^2-day) for the influent phosphate concentration of 20 mg/l

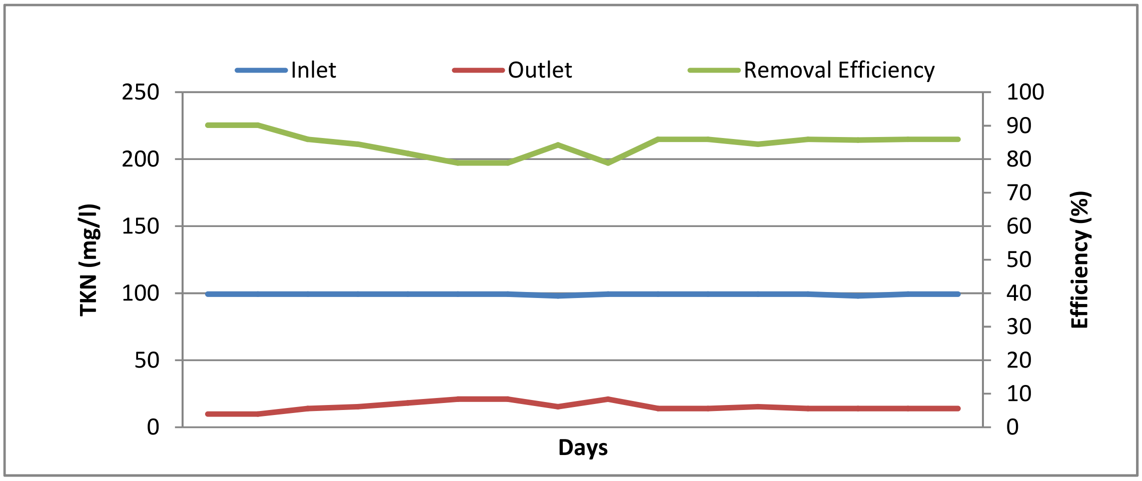

Figure 4.12: TKN concentration and its removal efficiency (%) for influent concentration of 100 mgN/l

4.7 Dissolved Iron

Iron is also present in wetland system which comes into water from wetland sediments and it was analysed to check whether the bed conditions of wetland are aerobic or anaerobic. Iron is found in two chemical forms i.e. Ferric (Fe^{3+}) representing oxidising conditions, and Ferrous (Fe^{2+}) representing the reducing conditions. Ferrous concentration in the present study varied from 0.57 mg/l to3.31 mg/l with an average of 2.27mg/l, against an average value of 0.19mg/l for Ferric ions. Ferrous constituted more than 90% fraction of total iron present in effluent. Therefore, reducing conditions dominated in the CW cell particularly at the depths. Presence of iron in reduced form rules out its binding with iron present in sediments and therefore the role of sediments in removal of phosphates by iron is very limited. Iron in the present case acts as Alternate Terminal Electron acceptor (ATEA), to oxidise the other chemical species present in wastewater. The result also confirms that the conditions are not completely anaerobic in the CW cell.

Table 4.11 Concentration of various iron fractions present in bed sediments

Date	**Concentration of iron fractions (mg/l)**		
	Ferrous (Fe^{2+})	**Ferric (Fe^{3+})**	**Ferric/ Ferrous ratio**
18-03-2014	3.312	0.397	0.12
19-03-2014	3.226	0.323	0.10
20-03-2014	3.199	0.054	0.02
21-03-2014	2.705	0.359	0.13
24-03-2014	2.633	0.467	0.18
25-03-2014	3.136	0.078	0.02
26-03-2014	2.829	0.030	0.01
27-03-2014	2.629	0.015	0.01
28-03-2014	3.146	0.102	0.03
31-03-2014	2.227	0.214	0.10
01-04-2014	2.482	0.081	0.03
02-04-2014	2.403	0.110	0.05

03-04-2014	1.642	0.052	0.03
07-04-2014	2.381	0.298	0.13
12-04-2014	1.437	0.216	0.15
13-04-2014	0.570	0.860	1.51
15-04-2014	1.672	0.045	0.03
16-04-2014	1.579	0.028	0.02
17-04-2014	1.425	0.137	0.10
18-04-2014	1.386	0.044	0.03
19-04-2014	1.875	0.089	0.05
20-04-2014	2.009	0.115	0.06
Range (Min-Max)	**0.57-3.31**	**0.015-0.86**	**0.01-1.51**
Mean ± S.D	**2.268 ±0.75**	**0.187 ±0.2**	**0.132 ±0.31**

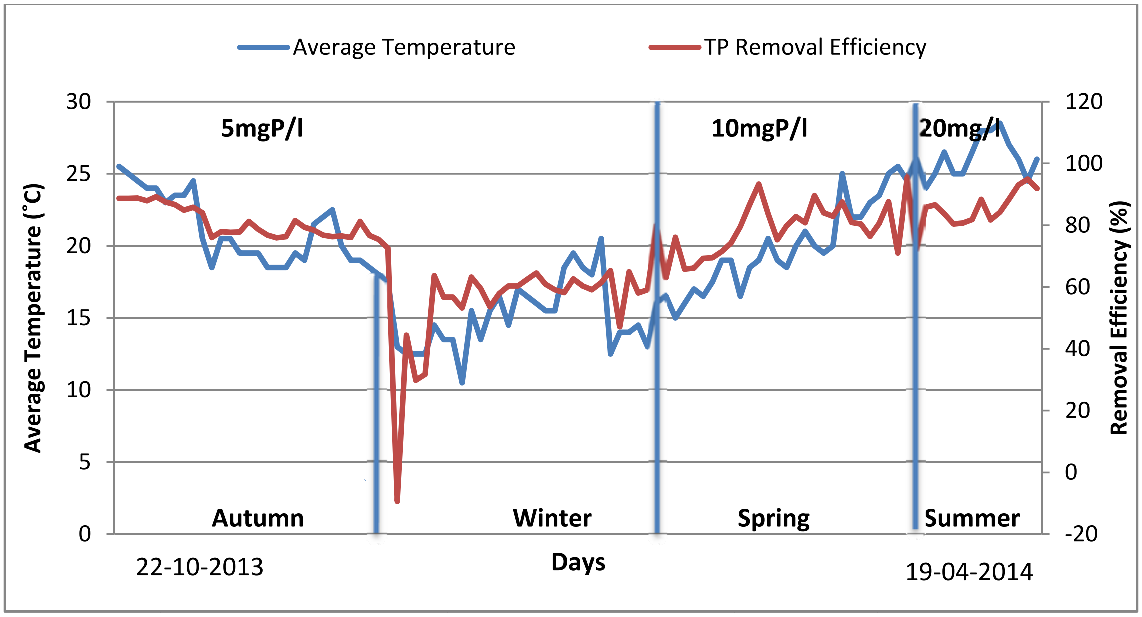

Figure 4.13 Removal efficiency (%) and average ambient temperature (^{o}C) during the study

CONCLUSION

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

1) *Brachaira mutica* can well adapt to the wetland condition under sub-tropical Indian environment and can easily absorb the chemical shocks with respect to the concentration of influent phosphate. It is important to mention that the influent should be rich in nitrogenous matter since deficiency of nitrogen superimposing with low temperature may exert a stress over the plant, as observed in the present study.

2) *The B. mutica* – based CW cell efficiently removes the nutrients (Nitrogen and Phosphorous), but an increase in TDS in effluent during treatment may be observed. Therefore the dissolution of various chemical species from the bed of CW cell is a cause of concern and the regulating factors maybe investigated.

3) The removal behaviour of *B. mutica* is largely dependent on the meteorological conditions, and chiefly on temperature and sunshine hours. Temperature regulates the rate of evapotranspiration, *i.e.,* higher the temperature more would be the rate of evapotranspiration, resulting in an increased acquirement/updraft of water along with nutrients. On the other hand, sunshine hours regulates photosynthetic rate and the productivity. More sunshine hours in summer result in higher productivity and increased uptake of nutrients. Therefore, removal efficiency for phosphate followed the trend as winter season(55.24%) < spring season (78.8%) < autumn season(80.7%) < summer season(85.6%) in the present sudy.

4) Removal efficiency of *B. mutica* in the present study followed first order kinetics as the influent concentration increased from 5mg/l to 20mg/l, removal efficiency went on increasing from 61.97% (TP) to 85.64%.

5) The conditions in the CW cell was observed to be nitrifying, *i.e.* oxidation of reduced form of nitrogen (NH_4^+) to nitrate removal of TKN from the influent was of the order of 75.6% (at 50mgN/l) and 84.6 (at 100mgN/l). Since the pH of effluent was around neutral throughout the study, transformation of NH_4^+ ammonia gas (NH_3) is ruled out as the nutrient loading is increased the conditions changed to reducing (at depths) gradually.

6) Stratification of CW cell with respect to oxygen was observed and it represented a clino grade oxygen profile. The surface water-sediment microzone represented aerobic (oxidising) conditions, whereas at depth reducing conditions

dominated. This resulted in a higher fraction (92%) of ferrous ions out of total iron concentration in the effluent representing dominance of reducing conditions in the CW cell.

7) The major removal of phosphate in the CW cell was by *Brachiaria*. Since the conditions were reducing binding of phosphates to Fe_2^+ ions does not take place and since the pH was almost neutral throughout the study, binding to calcium is ruled out (takes place at pH more than 9.0). therefore, binding of phosphates to sediment is negligible.

SUMMARY

The aim of the study was to monitor and study the removal rate and efficiency of phosphates as AP and TP. For this a bench scale *Brachiaria* based constructed wetland cell was prepared and subjected to wastewater. The system consisted of a bench-scale subsurface flow wetland (SSFW) built in laboratory at Delhi Technological University in which *Brachiaria mutica* was planted and grown. The CW cell had soil substrate packed with gravel-sand mixer and it consisted of a 35cm thick layer of soil substrate. Tanks were of dimension 80 cm wide and 110 cm long with the surface area of tank as 8800cm². The water depth of reactor was kept 5cm above the surface with an effective volume of *400l.* Before starting the study, the wetland system was flushed with water entering from the top and exiting from an outlet provide at the bottom of the system. There has had been various studies on CW using different plant species both, in India and abroad too. However there has been either limited or no studies on removal efficiency on a *Brachiaria* based CW cell. This was the main reason for selecting *Brachiaria* as the wetland vegetation.

The study aimed at studying the phosphate removal efficiencyof *Brachiaria* species in a twofold approach. One was to assess performance of the plat during different season, namely autumn, winter, spring and summer. The other aim was to study removal efficiency of plant at varying concentration of nutrients.

During the study, meteorological conditions were also observed and noted. The removal behavior of *B. mutica* is largely dependent on the meteorological conditions, and chiefly on temperature and sunshine hours. Temperature regulates the rate of evapotranspiration, *i.e.,* higher the temperature more would be the rate of evapotranspiration, resulting in an increased acquirement/updraft of water along with nutrients. On the other hand, sunshine hours regulates photosynthetic rate and the productivity. More sunshine hours in summer result in higher productivity and increased uptake of nutrients. Therefore, removal efficiency for phosphate followed the trend as winter season(55.24%) < spring season (78.8%) < autumn season(80.7%) < summer season(85.6%) in the present study. Hence it was concluded that *Brachaira mutica* can well adapt to the wetland condition under sub-tropical Indian environment and can easily absorb the chemical shocks with respect to the concentration of

influent phosphate with high removal rate. It is important to mention that the influent should be rich in nitrogenous matter since deficiency of nitrogen superimposing with low temperature may exerts a stress over the plant, as observed in the present study.*The B. mutica* – based CW cell efficiently removes the nutrients (Nitrogen and Phosphorous), but an increase in TDS in effluent during treatment may be observed. Therefore the dissolution of various chemical species from the bed of CW cell is a cause of concern and the regulating factors maybe investigated.Removal efficiency of *B. mutica* in the present study followed first order kinetics as the influent concentration increased from 5mg/l to 20mg/l, removal efficiency went on increasing from 61.97% (TP) to 85.64%.The conditions in the CW cell was observed to be nitrifying, *i.e.* oxidation of reduced form of nitrogen (NH_4^+) to nitrate removal of TKN from the influent was of the order of 75.6% (at 50mgN/l) and 84.6 (at 100mgN/l). Since the pH of effluent was around neutral throughout the study, transformation of NH_4^+ ammonia gas (NH_3) is ruled out as the nutrient loading is increased the conditions changed to reducing (at depths) gradually.Stratification of CW cell with respect to oxygen was observed and it represented a clino grade oxygen profile. The surface water-sediment microzone represented aerobic (oxidising) conditions, whereas at depth reducing conditions dominated. This resulted in a higher fraction (92%) of ferrous ions out of total iron concentration in the effluent representing dominance of reducing conditions in the CW cell.The major removal of phosphate in the CW cell was by *Brachiaria*. Since the conditions were reducing binding of phosphates to Fe_2^+ ions does not take place and since the pH was almost neutral throughout the study, binding to calcium is ruled out (takes place at pH more than 9.0). Therefore, binding of phosphates to sediment is negligible.

The plant can later be harvested and can be used as a fodder feed for horses and cows, thereby providing livelihood to people and source of food to animals. The plant can also be composted, as was done after the study was concluded, and the phosphate accumulated can serve as a source of organic manure for farms. The plant showed good growth and density, and being invasive species, it grows at a good pace, producing good biomass for composting or fodder feed, as the case may be.

Hence it can be stated with the above based result that the specie *Brachiaria mutica* can be used in CW cell for nutrient removal of high concentration wastewater with a good success rate. The CW cell does not require any significant operational or maintainance cost and can be used at places having tropical or sub tropical conditions.

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ANNEXURE-I

Date	Maximum Temperature in ° C	Minimum Temperature in ° C	Average Temperature in ° C	Rainfall Data (mm)
01-10-2013	34	25	29.5	**0**
02-10-2013	35	26	30.5	0
03-10-2013	34	23	28.5	0
04-10-2013	26	23	24.5	0
05-10-2013	34	22	28	0
06-10-2013	34	23	28.5	0
07-10-2013	34	24	29	0
08-10-2013	34	25	29.5	0
09-10-2013	36	25	30.5	0
10-10-2013	35	24	29.5	0
11-10-2013	28	21	24.5	0
12-10-2013	31	21	26	0
13-10-2013	33	23	28	0
14-10-2013	32	23	27.5	0
15-10-2013	33	21	27	0
16-10-2013	34	20	27	0
17-10-2013	35	21	28	0
18-10-2013	33	22	27.5	0
19-10-2013	34	18	26	0
20-10-2013	33	18	25.5	0
21-10-2013	32	17	24.5	0
22-10-2013	34	17	25.5	0
23-10-2013	32	18	25	0
24-10-2013	31	18	24.5	0
25-10-2013	30	18	24	0
26-10-2013	31	19	25	0
27-10-2013	30	18	24	0
28-10-2013	31	17	24	0

29-10-2013	31	15	23	0
30-10-2013	32	15	23.5	0
31-10-2013	30	17	23.5	0
01-11-2013	31	18	24.5	0
02-11-2013	29	16	22.5	0
03-11-2013	28	14	21	0
04-11-2013	28	13	20.5	0
05-11-2013	25	12	18.5	0
06-11-2013	28	13	20.5	0
07-11-2013	27	14	20.5	0
08-11-2013	25	14	19.5	0
09-11-2013	26	12	19	0
10-11-2013	26	12	19	0
11-11-2013	27	12	19.5	0
12-11-2013	27	12	19.5	0
13-11-2013	26	11	18.5	0
14-11-2013	26	11	18.5	0
15-11-2013	27	10	18.5	0
16-11-2013	27	9	18	0
17-11-2013	26	9	17.5	0
18-11-2013	27	11	19	0
19-11-2013	27	11	19	0
20-11-2013	28	10	19	0
21-11-2013	29	10	19.5	0
22-11-2013	27	11	19	0
23-11-2013	27	11	19	0
24-11-2013	28	12	20	0
25-11-2013	29	14	21.5	0
26-11-2013	29	15	22	0
27-11-2013	28	17	22.5	0
28-11-2013	28	12	20	0
29-11-2013	27	11	19	0
30-11-2013	27	12	19.5	0
01-12-2013	28	10	19	0

02-12-2013	28	10	19	0
03-12-2013	28	9	18.5	0
04-12-2013	26	10	18	0
05-12-2013	26	10	18	0
06-12-2013	25	10	17.5	0
07-12-2013	26	9	17.5	0
08-12-2013	26	10	18	0
09-12-2013	25	9	17	0
10-12-2013	24	9	16.5	0
11-12-2013	25	9	17	0
12-12-2013	24	9	16.5	0
13-12-2013	24	12	18	0
14-12-2013	25	10	17.5	0
15-12-2013	24	9	16.5	0
16-12-2013	24	9	16.5	0
17-12-2013	23	9	16	0
18-12-2013	22	9	15.5	0
19-12-2013	22	11	16.5	0
20-12-2013	20	12	16	0
21-12-2013	16	13	14.5	0
22-12-2013	17	12	14.5	0
23-12-2013	18	12	15	0
24-12-2013	20	10	15	0
25-12-2013	20	8	14	0
26-12-2013	21	6	13.5	0
27-12-2013	17	7	12	0
28-12-2013	20	5	12.5	0
29-12-2013	19	4	11.5	0
30-12-2013	20	2	11	0
31-12-2013	19	11	15	0
01-01-2014	18	2	10	0
02-01-2014	18	5	11.5	0
03-01-2014	20	5	12.5	0
04-01-2014	19	7	13	0

05-01-2014	19	8	13.5	0
06-01-2014	18	7	12.5	0
07-01-2014	20	6	13	4.4
08-01-2014	21	4	12.5	0
09-01-2014	18	7	12.5	0
10-01-2014	20	5	12.5	2.0
11-01-2014	18	7	12.5	0
12-01-2014	21	6	13.5	0
13-01-2014	22	7	14.5	0
14-01-2014	22	9	15.5	0
15-01-2014	17	10	13.5	0
16-01-2014	18	9	13.5	0
17-01-2014	13	8	10.5	6.0
18-01-2014	16	10	13	0
19-01-2014	21	9	15	0
20-01-2014	22	9	15.5	0
21-01-2014	15	12	13.5	0
22-01-2014	18	13	15.5	0
23-01-2014	20	13	16.5	0
24-01-2014	18	11	14.5	0
25-01-2014	14	9	11.5	0
26-01-2014	20	10	15	0
27-01-2014	24	10	17	0
28-01-2014	24	9	16.5	0
29-01-2014	22	10	16	0
30-01-2014	21	10	15.5	0
31-01-2014	21	10	15.5	0
01-02-2014	20	11	15.5	0
02-02-2014	20	7	13.5	0
03-02-2014	28	9	18.5	0
04-02-2014	26	13	19.5	0
05-02-2014	25	12	18.5	0
06-02-2014	26	10	18	0
07-02-2014	26	15	20.5	0

08-02-2014	17	11	14	0
09-02-2014	20	7	13.5	0
10-02-2014	20	5	12.5	0
11-02-2014	22	6	14	4.2
12-02-2014	21	7	14	0
13-02-2014	23	6	14.5	0
14-02-2014	15	11	13	26.2
15-02-2014	18	9	13.5	5.4
16-02-2014	20	7	13.5	0
17-02-2014	22	10	16	0
18-02-2014	23	10	16.5	0
19-02-2014	23	7	15	2.8
20-02-2014	24	8	16	0
21-02-2014	25	9	17	0
22-02-2014	22	12	17	0
23-02-2014	22	12	17	0
24-02-2014	23	10	16.5	0
25-02-2014	25	10	17.5	0
26-02-2014	26	12	19	0
27-02-2014	25	13	19	0.1
28-02-2014	21	12	16.5	8.6
01-03-2014	20	12	16	39.0
02-03-2014	22	10	16	0
03-03-2014	26	11	18.5	0
04-03-2014	26	12	19	0
05-03-2014	27	14	20.5	0
06-03-2014	26	12	19	0
07-03-2014	26	11	18.5	0
08-03-2014	27	13	20	0
09-03-2014	29	14	21.5	0
10-03-2014	24	16	20	2.6
11-03-2014	27	15	21	1.3
12-03-2014	27	13	20	4.2
13-03-2014	26	13	19.5	0

14-03-2014	28	12	20	0
15-03-2014	30	13	21.5	0
16-03-2014	32	16	24	0
17-03-2014	34	16	25	0
18-03-2014	30	20	25	3.6
19-03-2014	28	16	22	0
20-03-2014	29	15	22	0
21-03-2014	32	14	23	0
22-03-2014	32	17	24.5	0
23-03-2014	28	18	23	0
24-03-2014	29	18	23.5	2.4
25-03-2014	33	17	25	2.0
26-03-2014	32	19	25.5	0
27-03-2014	29	20	24.5	0
28-03-2014	33	19	26	8.1
29-03-2014	32	19	25.5	0
30-03-2014	31	18	24.5	0
31-03-2014	31	17	24	0
01-04-2014	33	17	25	0
02-04-2014	35	18	26.5	0
03-04-2014	32	18	25	0
04-04-2014	32	18	25	0
05-04-2014	35	20	27.5	0
06-04-2014	38	20	29	0
07-04-2014	32	21	26.5	0
08-04-2014	32	19	25.5	0
09-04-2014	33	18	25.5	0
10-04-2014	34	18	26	0
11-04-2014	36	17	26.5	0
12-04-2014	37	19	28	0
13-04-2014	33	23	28	0
14-04-2014	34	21	27.5	0
15-04-2014	36	21	28.5	0
16-04-2014	34	20	27	3.2

17-04-2014	32	20	26	0
18-04-2014	30	19	24.5	13.2
19-04-2014	32	20	26	0
20-04-2014	34	19	26.5	0
21-04-2014	36	19	27.5	0
22-04-2014	36	20	28	0
23-04-2014	36	22	29	0
24-04-2014	37	21	29	0
25-04-2014	39	21	30	0
26-04-2014	39	22	30.5	0
27-04-2014	38	21	29.5	0
28-04-2014	39	21	30	0
29-04-2014	41	23	32	0
Mean	**26.8**	**13.58**	**20.19**	**0.52**
S.D.	**±5.94**	**±5.33**	**±5.38**	**±3.20**