Efficacy of *Canna lily* and *Cyperus alternifolius* Towards the Removal of Phosphate from Wastewater Using a Constructed Wetland

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SUBMITTED BY:
NAMAN JAIN
(2K20/ENE/07)

RESEARCH SUPERVISOR:
PROF. A. K. HARITASH

DEPARTMENT OF ENVIRONMENTAL ENGINEERING

DELHI TECHNOLOGICAL UNIVERSITY

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DELHI TECHNOLOGICAL UNIVERSITY

(*FORMERLY* DELHI COLLEGE OF ENGINEERING)
MAIN BAWANA ROAD, NEW DELHI -110042

DEPARTMENT OF ENVIRORNMENTAL ENGINEERING

CERTIFICATE

This is to certify that the research work embodied in this dissertation entitled "**Efficacy of *Canna lily* and *Cyperus alternifolius* towards the Removal of Phosphate from Wastewater using a Constructed Wetland"** has been carried out in the Department of Environmental Engineering, Delhi Technological University, New Delhi. This work is original and has not been submitted in part or full for any other degree or diploma to any university or institute.

Dr. A.K Haritash
(Research Supervisor)

Naman Jain
(2K20/ENE/07)

Date:
Place:

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Date: **Naman Jain**
Place:

ABSTRACT

Considering the extensive eutrophication of water bodies, sustainable treatment techniques such as constructed wetlands (CWs) have emerged as a possible alternative for nutrient removal and wastewater treatment. The present study was undertaken to investigate the potential of *Canna lily* and *Cyperus alternifolius* based CW for removal of phosphate (PO_4^{3-}-P) in different seasons of a sub-tropical region. During the current study, the intake phosphate concentration was taken as roughly 5 mg/l. As a result, in the present study, removal efficiency for total phosphate (TP) in *Canna lily* followed the trend of winter season (77.56 %) < spring season (82.61 %) < summer season (87.79 %). The removal efficiency of *Cyperus alternifolius* CW cell was of order winter season (62.35 %) < spring season (74.24 %) < summer season (81.49 %). *C. lily* TP effluent concentrations throughout the study ranged from 0.18 mg/l to 1.82 mg/l while the average TP effluent concentrations in *C. alternifolius* ranged from 0.33 mg/l to 2.65 mg/l. The accumulation of (TP) in various parts of plant tissue were investigated in order to determine the overall effect on the plants during the study period. The concentration of PO_4^{3-}-P (mg/g) in roots, stems, leaves, and flowers has been analyzed. The effluent pH (6.2-8.3) was in the neutral range, and oxidising conditions in the rhizosphere ruled out the possibility of phosphate bonding with cations (Ca, Fe, and Al) in sediments. Evapotranspiration, plant metabolic activity, and hence nutrient removal were all influenced by ambient temperature and sunlight hours.

KEY WORDS: Phosphate, Eutrophication, Constructed wetland, *Canna lily*, *Cyperus alternifolius*, Wastewater.

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ABBREVIATIONS

AG	Analytical Grade
AP	Available Phosphate
APHA	American Public Health Association
CW	Constructed Wetland
CWS	Constructed Wetland System
DAP	Di-ammonium Phosphate
DO	Dissolved Oxygen
EC	Electrical Conductivity
EW	Engineering Wetland
FWS	Free Water Surface
G	Specific Gravity
HF	Horizontal Flow
HRT	Hydraulic Retention Time
SSF	Sub-surface Flow
SD	Standard Deviation
TDS	Total Dissolved Solids
TP	Total Phosphate
VF	Vertical Flow
VFCW	Vertical Flow constructed wetland

INTRODUCTION

Various studies have taken place in past in which natural or artificial wetland was used for wastewater treatment. A wetland is a region of land where the soil is either seasonally saturated or constantly saturated with moisture, giving rise to the characteristics of a distinct ecosystem. The potential of both natural and anthropogenic wetlands to purify wastewater is widely known. Wetlands cleanse the water through a complex number of physical, and chemical processes. In many cities, towns, villages and in other places of the world, wastewater from individual houses, small settlements, dwellings units and industries discharge their effluent into the nearby water body and thus indirectly into the environment with only poorly efficient primary treatment system or without any treatment at all which in return often poses a significant threat, danger and damage to the public and environmental health (Gardner *et al*., 1997). In order to limit the groundwater pollution, contamination and eutrophication of surface water bodies such as lakes, ponds and rivers, regulations for the discharge of treated wastewater have become stricter in recent years which makes nutrients such as phosphate and nitrate removal an increasingly common requirement for all small and decentralized wastewater treatment systems (Sasse, 1998; Wallace & Knight, 2006). The quality of ground water also improves in the vicinity of any wetland. The use of constructed wetlands (CW) for wastewater treatment has gained prominently across the world. As the application of CW, is to remove nutrients (phosphorus and nitrogen compounds) that are detrimental to the balance of more sensitive receiving water bodies such as lakes and ponds where it may result in eutrophication (Khan, 2014). Wetlands are cheaper to construct than any other conventional treatment option. Operation and maintenance costs (energy and supplies) are lower in the case of CW since it is a natural process and it does not require continuous monitoring. Skilled labour is also not required for the operation as well as in the monitoring. If fluctuation occurs during peak time or season, it does not affect the efficiency of the wetland system. However, this system will produce biomass that can be used for energy production in rural areas, can be used for thatching (Rajasulochana and Preethy, 2016). There are no foul odours or harmful by-products developed from this system. The plants also perform environmental service by fixation of carbon dioxide and production of oxygen.

The use and application of constructed wetlands (CW) for wastewater treatment has become common practice worldwide not only in developing countries but also in developed countries. A CW is a man-made wetland aimed to be used as new or restored habitat for native and migratory wildlife for human discharges such as wastewater, storm water runoff from nearby land area or sewage treatment, for land redemption after mining, refinery, and other ecological disturbances such as required relief for natural areas lost to a development. Wastewater treatment in a constructed wetland takes place as the water passes through the wetland medium in which plant grows and the plant rhizosphere (root zone of the plant). An aerobic thin film is formed around each root hair, as of results of the leakage of oxygen from the rhizomes and roots. Aerobic and anaerobic micro-organisms help in the breakdown of organic matter (Choudhary *et al*., 2011). The process of microbial nitrification followed by denitrification releases nitrogen gas into the atmosphere. Phosphorus is co-precipitated along with iron, aluminum and calcium compounds present in the root-bed medium. Suspended solids either filter out as they settle in surface flow wetlands or are manually filtered out by the medium within subsurface flow wetland cells. Harmful bacteria and viruses are decreased by filtration and adsorption on the rock media in subsurface flow wetlands (Choudhary *et al*., 2011)

Most of the research on constructed wetlands has been conducted in western countries, and very few were conducted in India. As a result, the plants which are primarily studied for treatment purposes include cattail (*Typha spp.*), bulrush (*Scirpus lacustris*) and reeds (*Phragmitis australis*), as these plants can sustain harsh winters. It is known that ornamental plants such as *canna lily* (*Canna flaccida*), umbrella sedge *(Cyperus alternifolius)*, elephant ear (*Colocasia sculenta*), ginger lily (*Hedychium coronarium*), and yellow iris (*Iris pseudacorus*) can be used in rock/plant filters to treat septic tank effluents (Wolverton, 1990). Among the floating aquatics, water hyacinth (*Eichhornia crassipes*) and duckweeds (*Lemna spp.*) have been studied most extensively for nutrients uptake efficiency. However, not much is documented about the efficiency of these ornamental plants for nutrient removal from wetlands. The fact that these ornamental plants cannot survive the cold winters in northern countries, hence, that could be one main reason why they have had limited use in constructed wetlands (Belmont *et al*., 2003). Since the majority of developing countries are in tropical and subtropical areas and have inadequate resources to implement conventional wastewater treatment systems, the use of ornamental plants in treatment wetlands should be investigated.

Both *Canna lily* and *Cyprus alternifolius* have been discovered as the suitable candidates towards the removal of nutrients without compromising its treatment efficiency and aesthetics (Haritash *et al.*, 2017). There haven't been many studies on nutrients (specifically phosphorus) removal efficiency. Keeping this fact in mind, the present study will be mainly focused on phosphorus removal efficiency using *Canna lily* and *Cyprus alternifolius* in a constructed wetland. In this study, the influent which was supplied to the plants was synthetically prepared in the laboratory. Another important objective of this study is to get an idea about the various fraction of phosphorus present in wastewater effluent.

Since, phosphate removal efficiency will depend upon these macrophytes (*Canna lily* and *Cyprus alternifolius*), and more particularly, phosphate uptake efficiency, will depend upon accumulation of phosphate in various parts of plant tissue. This study will also include this aspect, and a brief comparison study has been done to know the effect on translocation of phosphate in different parts of the plant under this study.

Phosphate (PO_4^{3-}-P), being a major growth limiting nutrient in the biosphere, usually plays a key role in governing the biological productivity of aquatic ecosystems (Worsfold *et al*., 2008). Eutrophication due to elevated PO_4^{3-} inputs to the aquatic environment which increases the risk of algae blooms and impact on water quality. The main sources of phosphate in lakes and ponds are external point and non-point sources such as rainfall runoff, soil leaching, industrial and municipal effluents etc.

This study is designing such a way that it will meet the following objectives:

- To study the phosphate removal efficiency of *Canna lily* and *Cyperus alternifolius* based CW.
- To study the translocation of phosphate in macrophyte.
- To study the growth of *Canna lily* and *Cyperus alternifolius* for different weather during this study.
- To suggest the efficacy of both macrophytes for phosphate removal efficiency from wastewater.

REVIEW OF LITERATURE

2.1 Phytoremediation

Phyto-remediation is a process in which the term Phyto means plant and remedy means to restore or to cure. Thus, phytoremediation refers to the treatment and cure of environmental problems using plants that help in reducing the problem without even excavating the contaminant material and disposing it off elsewhere.

Engineering wetland (EW) phytoremediation system consists of mitigating pollutant concentrations in contaminated soils, water or air, with the help of plant which are able to degrade or eliminate nutrients such as phosphate, poly-aromatic hydrocarbons, landfill leachate, metals and heavy metals, pesticides, solvents, explosives, crude oil and their derivatives, and various other contaminants that contain them (McCutcheon *et al*., 2008) can be constructed artificially as a water management tool, often called as constructed wetland (CW). The largest natural wetlands in the world are the Amazon River basin. A constructed wetland (CW) is an artificial swamp designed and constructed to inculcate the natural processes like wetland vegetation, soils and their associated microbial activities to aid and assist in waste treatment (Richardson, 2001).

Plants in every natural wetland provide a substrate upon which microorganisms can grow as they break down organic materials and uptake nutrients and heavy metals (McCutcheon *et al*., 2008). There has been alarming increase in the demands of human expansion and resources exploitation in present. Thus, the natural wetland systems in such cases cannot always function efficiently as per requirement, and strict and stringent water quality standards. This may be the factor which led to the rapid development of constructed wetlands (CW) for wastewater treatment.

Efficiency of CWS might be less consistent as compared to conventional treatments due to the environment changes occurring in different seasons. The biological components are sensitive to shocks caused by toxic chemicals, e.g., ammonia and pesticides. Pollutants or surges in water flow can temporarily hinder the treatment process. Thus, engineered wetlands

(EWSs) are designed to incur for the limitations of CWS in a more controlled way (Davis, 2009). Engineered wetlands (EW) are advanced and semi-passive kind of CWs in which operating conditions are monitored, manipulated and controlled in a more effective manner so that contaminant removal is optimized. With EW many kinds of biological and chemical processing systems, e.g., aerobic and anaerobic bioreactors, limestone drains can be expressed as cells of the system. EW can be used to bridge the gap between active treatment and the passive treatment in CW, ending the need for more engineered treatment methods and technologies (Higgins *et al*., 2000).

2.2 Essential Nutrients

Plants and animals require nutrients to flourish and survive, which are chemical substances contained in food. Despite the fact that there are other nutrients, nitrogen and phosphorus are two of the most essential and abundant. Nitrogen and phosphorus come in many different forms, or species, and the species present can vary as they migrate through the air, water, and soil (Frank *et al*., 2003).

Sources of Nutrients

The following are the most common causes of excess nutrients (nitrogen and phosphorus) (Badruzzaman *et al*., 2012):

- **Agriculture**: Crops require nitrogen and phosphorus, which can be found in animal dung and commercial fertilizers like diammonium phosphate (DAP). When these nutrients are not fully absorbed by plants, they can be lost from farm fields and dispersed through dust and surface runoff, posing a threat to air and water quality downstream.
- **Stormwater**: When rain falls on our cities and towns, it flows through hard surfaces such as rooftops, sidewalks, and roadways, carrying pollutants such as nitrogen and phosphorus into nearby rivers.
- **Wastewater**: Our sewage and septic systems are in charge of handling vast amounts of waste, and they don't always work effectively or remove enough nitrogen and phosphorus before being discharged into rivers.
- **Fossil Fuels**: The usage of fossil fuels has increased the rate of nitrogen in the air due to electricity generation, industry, transportation, and agriculture.

- **In and around the home**: Fertilizers, yard and pet waste, and some soaps and detergents all contain nitrogen and phosphorus, and if not utilized or disposed of appropriately, they can contribute to nutrient overload. During wet conditions, the density of hard surfaces and the kind of landscaping can further enhance nitrogen and phosphorus runoff.

2.3 Why is it important to evaluate phosphorus?

Phosphorus is an essential component for all lifeforms. Phosphate (PO_4) is the most frequent type of phosphorus utilized by biological organisms, and it is essential in the production of DNA, cellular energy, and cell membranes (and plant cell walls). In commercial fertilizers, phosphorus is a prevalent element (Wang *et al*., 2009).

Poor agricultural activities, runoff from urban areas and lawns, leaking septic systems, and sewage treatment plant outputs can all cause high phosphorus levels. Too much phosphorus can promote the growth of algae and massive aquatic plants, lowering dissolved oxygen levels — a process known as eutrophication. Phosphorus overload can also cause algae blooms, which create algal toxins that are detrimental to human and environmental life (US EPA, 2015; Pretty, *et al*., 2003).

In an aquatic ecosystem, phosphorus is often referred to as the "limiting nutrient," meaning that the amount of this nutrient available regulates the rate at which algae and aquatic plants are grown (Correll, 1999). Phosphorus may be utilized by plants and soil microorganisms for proper development in adequate amounts. Excess phosphorus, on the other hand, can cause water quality issues such as eutrophication and toxic algae blooms (US EPA, 2015; Pretty, *et al*., 2003). Some aquatic resources, such as wetlands, act as natural sinks for phosphorus dissolved in water or found in sediments. However, because phosphorus is found in small amounts in nature, even minor increases can have a severe impact on water quality and biological condition.

2.4 Effects of Eutrophication

- **Hinder fish and other sea life forms' survival.**

Algal blooms occur when nutrients in aquatic ecosystems are augmented, causing phytoplankton and other photosynthesis plants to grow rapidly. As a consequence, algal blooms reduce the amount of dissolved oxygen (DO) available for other aquatic animal and plant species to consume. When algae/plant life dies and decomposes, oxygen is depleted.

Animal and plant species under the water, including as shrimp, fish, and other aquatic biota, choke to death when the dissolving oxygen exceeds hypoxic levels. Anaerobic conditions can promote the growth of bacteria that produce toxins that are fatal to marine mammals and birds in extreme circumstances. This can result in aquatic dead zones and a decline in biodiversity (Wu, 1999).

- **Deterioration of water quality and limits access to safe drinking water**

Algal blooms are extremely hazardous, and as the water reaches anaerobic conditions, additional deadly bacteria thrive. As a result, water quality has deteriorated significantly, and the availability of safe drinking water has decreased. The dense growth of algal blooms and photosynthetic bacteria in surface waterways can clog water systems, decreasing the amount of piped water available.

- **Poisoning and its consequences on human health**

Red tide is caused by cyanobacteria, also known as dinoflagellates, which release very potent toxins with high toxicity levels in the water, even at very low quantities. The anaerobic conditions caused by explosive plant growth in the water also cause the hazardous chemicals to double. When ingested in drinking water, it can harm humans and animals even at very low concentrations. Algal blooms in freshwater can be harmful to cattle. Toxic substances can also work their way up the food chain, causing a variety of health problems, including cancer.

Biotoxins have been related to an increase in neurotoxic, paralytic, and diarrhetic shellfish poisoning in humans, which can be fatal. The poison is stored in the mussels of the shellfish, which then poisons humans when they eat them. The ability of high nitrogen concentrations in drinking water to restrict blood circulation in neonates is linked to the disease known as blue baby syndrome.

- **Effect on Fish**

As previously stated, increased growth of small floating plants such as algae and cyanobacteria, as well as the development of broad and dense mats of floating plants such as Nile cabbage and water hyacinths, are two of the key aspects of eutrophication. Fishing is compromised whenever this occurs on a water body. It's simply more difficult to set fishing nets in water, and the floating plants restrict boat and other fishing craft maneuverability.

- **Deterioration of recreational opportunities**

The main aspect of eutrophication is algal blooms and other aquatic plants that float on a large area of the water surface. It lowers the water transparency and navigation, reducing the recreational value and potential of the lakes, particularly for boating and swimming. Nile cabbage, algal blooms, and other algae can grow over a large region along the beaches and even float over the entire surface into land areas.

2.5 Stages of eutrophication in lakes

Lakes have been shown to possess various zones of biological activity, which are mostly influenced by light and oxygen available. The biological zones that are most significant include:

- **Oligotrophic lakes:** Due to the low nutrient level in the lake, an oligotrophic lake has a relatively low production. Because algae growth is limited in these lakes, the water is usually clear. Such lakes provide water that is safe to drink. Such lakes support aquatic species who require well-oxygenated, cold such as lake trout. Because of the low temperatures of the lake waters, oligotrophic lakes are typically located in colder parts of the planet, where nutrient mixing is infrequent and slow (Gunn *et al.*, 2001).

- **Mesotrophic lakes:** These lakes are in the process of transitioning from oligotrophic to eutrophic. During summer stratification, there is some reduction in oxygen concentration in the hypolimnion (Gunn *et al.*, 2001).

- **Eutrophic lakes**: Eutrophic lakes have high levels of biological production, and the rich nutrient composition of this lake, particularly nitrogen and phosphorus, supports a plethora of plants. Due to the high quantities of oxygen provided by a large number of plants growing in the lake, eutrophic lakes initially stimulate the multiplication and expansion of Lake Fauna (Gunn *et al*., 2001). Plants or algal blooms overcrowd the lake, and the lake fauna suffers as a result of the high levels of respiration by the living vegetative matter. Eutrophication can happen naturally or as a result of human activity in the environment.

- **Hypereutrophic lakes:** Due to a rich supply of growth nutrients, these lakes have issues with excessive plant and algae growth. Because to the excessive overgrowth of algae or aquatic plants, these lakes exhibit little transparency. The visibility in these lakes is less than 3 feet. Phosphorus concentrations of more than 100 mg/l and total chlorophyll concentrations of more than 40 mg/l are also seen in hypereutrophic lakes (Gunn *et al*., 2001). Algal overgrowth frequently suffocates wildlife below the water's surface, resulting in dead zones under the water's surface.

- **Dystrophic lakes:** These are organic-rich lakes (humid and fulvic acids) that are fed by the lake's external inputs (watershed).

Table 2.1 Tropic bands for standing waters

Trophic Band	**Total phosphorus (mg/l)**
Dystrophic	< 0.005
Oligotrophic	0.005-0.01
Mesotrophic	0.01-0.03
Eutrophic	0.03-0.1
Hypertrophic	>0.1

Source: https://www.open.edu/openlearn/mod/oucontent/view.php?printable=1&id=2317

2.6 Wetland Systems

Wetlands can be broadly classified as:

a) Natural wetland

b) Constructed Wetland

(a) Natural Wetlands

Natural wetlands are completely or partially flooded. They get their water from the groundwater or from surface runoff. These are low-lying areas where the water table is above ground level, making the water supply perpetual. Environmental engineers have recognized their significant potential for filtration and treatment of contaminants, and they have successfully utilized them for this purpose.

(b) Constructed wetlands

The constructed wetland system (CWS) is a wastewater treatment system that works in carefully regulated situations to replicate the occurrences of soil, plant, and microbes in natural wetlands. Constructed wetlands (CW) provide a platform for testing flow regimes, micro-biotic compositions, and flora to develop the most efficient treatment technique. Hydraulic retention time (HRT) and hydraulic channels are also controlled (Brix, 1994). The water flow mechanisms along with the growth of flora are the most important elements of manmade wetlands. Surface flow systems with only free-floating macrophytes, floating-leaved macrophytes, or submerged macrophytes are all examples of CW systems (Vymazal *et al*., 2008). However, emergent macrophytes are commonly used in free water surface systems. CW can be used to treat raw sewage, secondary domestic sludge, oxidation pond discharge, storm waters, mining waste, and industrial and agricultural waste effluents, as well as to improve the water quality of oxidation pond discharge, storm waters, and mining waste. CW is made up of a cascade of rectangular or irregular-shaped wetlands cells that work on gravity force and are constrained by clay, rock, concrete, or other material boundaries. The flow regimes of CW for wastewater treatment can be categorised. A manmade wetland system can use three different types of cells (CWS):

1) Surface flow wetland cell
2) Sub-surface flow (SSF) wetlands
3) Hybrid wetlands

Surface -flow wetlands

Surface-flow wetlands, in which effluent moves above the soil level in a planted marsh or swamp, can sustain a larger range of soil types, including bay mud and other silty clays. Plants including cattails (*Typha*), sedges, Water Hyacinth (*Eichhornia crassipes*), and Pontederia are often employed in European created wetlands, despite the fact that *Typha* and *Phragmites* are very invasive. These wetlands were engineered and designed to take advantage of natural processes in the wetland systems, such as wetland vegetation, soils, and the microbiological activities that go along with them. It can be further divided into free-floating, floating-leaved, emergent, and submerged macrophytes based on the life forms of the dominant macrophytes in the system. In free water surface (FWS) environments, CWs with emergent macrophytes are the most prevalent. FWS CW are found all over the world, with the predominance in Europe. They've been used to treat municipal and domestic sewage, wastewater from livestock operations, industrial wastewaters, including agro-industrial wastewaters, and landfill leachates all over the world. FWS CWs have been proven to be a viable alternative to traditional treatment technologies in thousands of applications. (Vyzmazal, 2005).

Subsurface-flow wetlands

Subsurface-flow wetlands are those in which effluent from the household, agricultural, paper mill or mining runoff, tannery or meat processing wastes, or storm drains, or water from other sources to be cleansed, permeate through gravel composed primarily of limestone or volcanic rock lava stone or sand medium in which plants are rooted (Choudhary *et al*., 2011). Horizontal flow (HF) and vertical flow (VF) built wetlands are two types of subsurface-flow wetlands. The effluent in the packing medium in subsurface-flow systems can migrate horizontally, parallel to the surface, or vertically, from the planted layer down through the substrate and out. Because there is no water exposed to the surface in subsurface horizontal-flow wetlands, mosquitoes have a harder time breeding. Although subsurface-flow systems have the benefit of requiring less space for water treatment, they are not as good for wildlife habitat as surface-flow artificial wetlands.

For decades, constructed wetlands with horizontal subsurface flow (HF CWs) have been used to remediate wastewater. The majority of HF CWs were created to treat municipal or household wastewater. Municipal HF CWs concentrate not only on the removal of common contaminants through treatment, but also on specific criteria such as pharmaceuticals, endocrine disrupting chemicals, and detergent-derived linear alkylbenzensulfonates (LAS).

Many other types of wastewaters are treated with HF CWs; therefore, they have industrial applications as well. These include effluent from oil refineries, chemical plants, pulp and paper mills, tanneries and textile mills, as well as distilleries and wineries. HF CW is increasingly being used to treat food-processing wastewater, including as the production and processing of milk and dairy, cheese, potatoes, sugar, and other foods. HF constructed wetlands have also been used to treat wastewater from pig and dairy farms, fish farm effluents, and different runoff water from agriculture, airports, highways, greenhouses, and plant nurseries. HF CWs have also been utilized to efficiently remediate landfill leachate. In addition to being employed as a single unit, HF CWs are often used in hybrid systems with other forms of artificial wetlands. (Vymazal, 2009).

Hybrid wetlands

Surface and subsurface flows are integrated in these systems, which are also known as combined systems. Hybrid systems combine various types of built wetlands to improve treatment efficiency. Hybrid systems are made up primarily of vertical flow (VF) and horizontal flow (HF) systems that are staged. Because of their low oxygen transfer capability, horizontal flow systems can provide anaerobic conditions. In these systems, vertical flow systems do produce anaerobic conditions. The benefits of both the HF and VF systems can be used in hybrid systems to complement operations in each system to generate an effluent that is low in BOD and nitrogen. (Vymazal, 2005).

2.6 Wetland Vegetation

The potential of aquatic macrophyte dominated ecosystems to uptake and decompose nutrient and organic matter input has led in the widespread usage of such systems to treat various types of waste water. Waste water treatment systems based on aquatic macrophytes may be classified into: -

- Floating Macrophyte Treatment Systems
- Submerged Macrophyte Treatment Systems
- Emergent Macrophyte Treatment Systems

There are various studies of aquatic macrophyte based wastewater treatment systems in which natural or artificial wetland is used for water treatment. Wetlands are considered to have unique ecological features which provide numerous products and services to humanity Wetlands are considered to have unique ecological features which provide numerous products and services to humanity (Prasad *et al*., 2002). The particular vegetation that is adapted to its unique soil conditions that’s what separates wetlands from other land types or water bodies (Prasad *et al*., 2002). Wetlands are mainly composed of hydro soil, which supports aquatic vegetation. Wetlands may contain saltwater, freshwater, or brackish water. Swamps, marshes, bogs, and fens are the most prevalent forms of wetland (Keddy and Paul, 2010). Wetlands regulate terrestrial and aquatic ecosystems by cycling sediments and nutrients. The absorption and storage of nutrients available in the surrounding soil and water is a natural function of wetlands species. These nutrients are stored in the plant's system until it dies or is consumed by animals or humans. Wetland systems are extremely efficient and effective in retaining nutrients and trapping silt, yet each system has its own boundaries. An oversupply of nutrients from fertilizer run-off, sewage effluent, or non-point pollution causes eutrophication. Water flow, area, temperature, and plant species all influence the ability of wetland plants to acquire heavy metals.

Wetlands effectively remove aquatic pollutants from the environment via a complex set of biological, physical and chemical processes (Gersberg *et al*., 1986). When plants convert the complex substances into simpler form by enzymatic secretion or any other process like oxidation or reduction it is termed as phytoremediation. Phytoremediation is the remediation of environmental concerns by using plants to minimise the problem without excavating the toxic material and disposing of it elsewhere

2.6.1 Macrophytes

A plant community is a group of plants that naturally grow together in a certain habitat. The composition of a plant community is determined by a complex combination of factors such as climate, soil type, location in the landscape, and plant species competition. Wetlands are dynamic habitats with natural water level and water quality variations. As a result, certain wetland plants may survive both floods and brief periods of drought in the same year.

Different water-tolerant plant species are used in wetlands treatment systems. Surface flow (SF) wetlands and subsurface flow (SSF) wetlands are the two primary kinds of CW treatment systems based on flow type. Wetland vegetation is classified into three types.

- **Emergent Aquatic Macrophytes** are plants that live fully submerged, float, or have a little section of their body above the water's surface. They might be connected (for e.g *Canna lily*, Typha).

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

- **Submerged aquatic macrophytes** photosynthetic tissue is completely submerged, although the flower is generally exposed to the atmosphere.

Canna lily

Canna (or *Canna lily*, but not an actual lily) is a flowering plant genus with nineteen species. Cannas closest family members include the *Zingiberaceae* (gingers), Musaceae (bananas), *Marantaceae, Heliconiaceae, Strelitziaceae*, and other plant families of the order Zingiberales. Canna is the only genus in the Cannaceae family. Biologists almost universally acknowledge such a family. The family is also recognised by the APG II system of 2003, and is assigned to the order Zingiberales in the clade commelinids, in the monocots. Originally indigenous of South America, *Canna* (or *Canna lily*) is now distributed worldwide, especially in tropical and subtropical regions. Various local names that were given to this plant show how well spread and used this species is. It is called Achira in Spain, Balisier in French, Biri or Cana in Portugal, Queensland root in Australia, Sagu in Thailand, Chisqua in Columbia, Ganyong in Indonesia, to name a few. One of the reasons this species is well spread is that all parts of this plant are beneficial.

The rhizome can be boiled or processed to make flour, pasta, and a variety of snacks. The young leaves are eaten as a vegetable. The seeds are used as an ingredient in tortilla mixtures, and the stems and older leaves are used as animal feed. As a food source, *C. lily* is the best-known edible canna among its congeneric and provides a high amount of carbohydrates that could be used as an alternative to rice. The starch-rich rhizomes of *C. lily* contain about 94–96% starch, 0.05– 0.20% protein, 0.01–0.15 % fat, 0.40–0.90% fiber, and 0.70–0.90% ash per dry weight basis (Piyachomkwan *et al*., 2002). The leaves and roots of this plant also have pharmaceutical benefits that have been used as home remedies for many generations. Another reason for its wide distribution is its soil conservative characteristics *C. lily* is a fast-growing species producing large leaves that are capable of protecting the soil from the impact of direct rainfall.

This species also survives well on dry and marginal soils. With the continuous increase in the human population and rapid degradation of forested areas for agricultural production, it is just a matter of time before a shortage of food occurs in the near future *C. lily* has the potential to be used as a food crop, hence, to help to solve the world food crisis while conserving the soil.

Fig. 2.1 *Canna lily*-based CW used during the study.

Due to its thick, impenetrable seed coating, Canna is the only member of the Liliopsida class (monocot group) in which seed hibernation is known to occur. Cannas thrive in full sun and moderate water, as well as well-drained rich or sandy soil. Ribosomes can be dug out before freezing and kept in a safe space (above 7°C) in areas where the temperature drops below roughly -10°C in the winter. *C. lily* should be planted in full sun or almost full sun, away from scorching walls or reflected light. They also perform well in very light shade in our environment, but not in deep shadow.

Cyperus Alternifolius

Cyperus alternifolius is a member of the Cyperaceae family. It is endemic to Africa's north and tropical regions. *C. alternifolius*, considered from semi aquatic plants and with the common names of umbrella plant, umbrella papyrus, and umbrella sedge or umbrella palm. (Bailey, 1962). Umbrella papyrus is now used primarily as graceful accent in water gardens and at the margins of pools or ponds. It has a valuable usage as wonderful unusual cut flower (Hasegawa *et al*., 1998b) and in a wide variety of landscaping i.e., tropical looking accent plant, patio and pot plant (Hasegawa *et al*., 1998a). Also, it used in sewage treatment, as well as the reclamation of contaminated land with heavy metals. (Jones and Humphries 2002).

Fig. 2.2 *Cyperus alternifolius* based CW used during the study.

Water Hyacinth

Water hyacinth (WH) is a free floating, perennial aquatic plant originated from Amazon River basin. Its leaves are broad, thick and glossy. Scientific name of water hyacinths is ***Eichhorina crassipes***. This type of plant may rise 1 m above the surface of water. Leaves are

10-20 cm across, and float above the water surface. The feathery, freely hanging roots are purple-black. It has exhibited extremely high growth rates and the coverage of waterways by WH has created several problems including destruction of eco systems, irrigation problems and also as a mosquito breeding place leading to increase in mosquito population (Sornvoraweat and Kongkiattikajorn, 2011). It is the most productive plant on the planet, yet it is also a severe danger to biodiversity. These harmful consequences of WH prompted a slew of research and development projects aimed at eradicating this infamous plant. Controlling this plant comes at a significant expense and requires a lot of labour. For the control and eradication of WH, several biological, physical, and chemical treatments have been explored, but none of these measures has shown to be a long-term answer.

Water hyacinth can reduce phytoplankton productivity, dissolved oxygen, nitrogen, phosphorus, heavy metals, and other pollutant concentrations while also altering the quality of the water. When water hyacinth is present in moderate abundance, a rise in macroinvertebrate and fish richness and diversity suggests a possibly good relationship with waterbirds.

WH has a cellulose content of about 20%, a hemicellulose content of 48%, and a lignin content of 3.5 percent. Because of its great productivity, it could be used as a feedstock for biofuel production. WH offers various advantages, including the ability to grow on water without competing with arable land for grain and vegetable production. Several reports are available for the conversion of WH to fuel ethanol and biogas (Okewale *et al*., 2016; Gunja *et al*., 2016; Das *et al*., 2016; Shah *et al*., 2015).

Fig. 2.3 Water hyacinth macrophyte

Typha

The genus Typha spp. is usually referred to as the “cattail genus.” Three species of this genus, *Typha latifolia* L. (common cattail or broadleaved cattail), *Typha angustifolia* L. (narrow leaved cattail), and their hybrid, *Typha x glauca* (hybrid cattail) are widespread throughout North America (Grace and Harrison 1986).

Typha spp. is an erect, rhizomatous, perennial aquatic herb which bears flowers on a slender stem 1 – 3 m tall. The minute flowers, clustered together into spikes, form a cylindrical inflorescence with the staminate (male) flowers located above the pistillate (female) flowers (Stevens and Hoag, 2000). The reddish to blackish-brown staminate flowers can be 7 to 13 cm long, whereas the dark brown pistillate flowers grow between 2.5 to 20 cm long (Mitich 2000; Grace and Harrison 1986; Motivans and Apfelbaum 1987). Creeping lateral rhizomes, also known as underground stems, grow up to 70 cm long and 0.5-3 cm wide from the base of the plant's leaves. The plant’s grayish, green leaves are flat, linear, and long, often overtopping flowering spikes (Mitich 2000; Grace and Harrison 1986; Motivans and Apfelbaum 1987).

Cat tail colonies can be found all over the world, in both humid and dry climates, from tropical to temperate zones. Their tolerance to varying climatic conditions and environmental changes helps them achieve widespread dominance in a variety of aquatic plant communities (Mitich 2000; Murkin and Ward 1980). Cattails can occur in any place where the soil remains wet or saturated: roadside ditches, reservoirs, lakeshores, bogs, wet meadows, marshes, etc. (Grace and Harrison 1986). Although the cattail is a freshwater aquatic plant, it can tolerate some degree of salinity and acidity (Grace and Harrison 1986). It is also tolerant of perennial flooding, poor soil conditions (Stevens and Hoag 2000), and high concentrations of lead, zinc, copper, and nickel (Motivans and Apfelbaum 1987). *Typha latifolia* can be found in relatively undisturbed habitats, whereas Typha angustifolia typically occurs in more unstable and saline environments (Grace and Harrison 1986). According to Wilcox et al. (1984), *Typha angustifolia* is restricted to deeper waters and more salty environments when the two species are found together. In shallow water, the *Typha latifolia* thrives. Their hybrid species, Typha x glauca, has similar habitat requirements to *T. angustifolia* (Motivans and Apfelbaum 1987). *Typha latifolia* can be found in aquatic communities at all stages, from early to late successional, whereas *T. angustifolia* and T. *x glauca* typically occur in early to mid-successional communities and are frequently found in disturbed wetland sites (Grace and Harrison 1986). All Typha spp. species, according to Grace and Harrison, can be found in dense monospecific stands, scattered individuals, or clumps in mixed vegetation stands (1986). Typha spp. is often surrounded by or intermixed with other plants such as *Phragmites australiis*, *Lythrum salicaria*, *Spartina sp.*, *Acorus calamus*, *Scripus sp.*, and *Sagittaria latifolia* (Motivans and Apfelbaum 1987).

Fig 2.4. Cat tail macrophyte

2.8 Contaminant removal mechanism in the Constructed Wetlands

Wetland can effectively remove or convert vast quantities of pollutants, including organic matter, suspended particles, trace metals, and nutrients, from point sources (municipal, industrial, and agricultural wastewater) and non-point sources (mines, farmland, and urban runoff). The goal of wastewater treatment by artificial wetlands is to maximise microbial species contact with the substrate, with the ultimate goal of bioconversion to carbon dioxide, biomass, and water. Wetlands are characterized by a range of properties that make them attractive for managing pollutants in water (Bavor & Adcock, 1994). High plant production, big sediment adsorptive capacity, high rates of oxidation by micro flora associated with plant biomass, and a substantial buffering capacity for nutrients and pollutants are some of these qualities.

- **Physical processes:** The significant physical processes that lead to the disposal of wastewater contaminants are sedimentation and filtration. Water residence time affects the efficacy of all processes (biological, chemical, and physical) (i.e., the length of time the water stays in the wetland). Longer retention times speed up the removal of more pollutants, but they can also have negative consequences.

- **Chemical processes:** Metals can precipitate as insoluble compounds from the water column. Exposure to light and atmospheric gases can break down organic pesticides, or kill disease producing organisms (EPA, 1995). The direction of many reactions and activities in wetlands is influenced by the pH of water and soils, including biological transformation, partitioning of charged and uncharged forms of acids and bases, cation exchange, and solid and gas solubility.

- **Biological processes**: There are six major biological reactions involved in the performance of constructed wetlands, including photosynthesis, respiration, fermentation, nitrification, de-nitrification and microbial phosphorus removal (Mitchell, 1996). Wetland plants and algae perform photosynthesis, which adds carbon and oxygen to the ecosystem. The nitrification process is fueled by both carbon and oxygen. Plants carry oxygen from their leaves to their roots, where it enters the root zones (rhizosphere). Respiration is the oxidation of organic carbon by all living creatures, which results in the production of carbon dioxide and water. Bacteria, fungus, algae, and protozoa are the most common microorganisms found in the CW. The effective functioning of wetland species requires the maintenance of ideal circumstances in the system.

- **Removal mechanism of Suspended Solids:** Surface flow (SF) constructed wetlands are particularly effective in removing suspended particles. Flocculation/ sedimentation and filtering are the most common physical processes for removing suspended particles (EPA, 2000; Kadlec, 2009). Various types of pollutants, such as nutrients, heavy metals, and organic compounds, can be found in suspended matter in wastewater (Debusk, 1999). In wetland systems, microbial growth aids in the removal of colloidal particles. When the growth of vegetation in a particular wetland system is more the removal efficiency of suspended solids and colloidal solids is more. Surface forces such as Vander Waal's force of attraction and electric forces, which can be either attracting or repulsive depending on the surface charges, are also responsible for the reduction of suspended solids (Metcalf and Eddy, 1991).

- **Removal mechanism of Heavy Metals / Contaminants:** The main mechanism for removing metals from industrial wastewater in constructed wetlands (Stottmeister et al., 2003; Debusk, 1999) as follows:

(a) Filtration and sedimentation
(b) Precipitation
(c) Adsorption
(d) Uptake by the macrophytes and microorganisms

(a) **Filtration and sedimentation**: are the primary method for removing heavy metals from waste water in CWs. Cadmium, lead, silver, and zinc were removed by filtration, according to Sinicrope et al. (1992) and Noller et al. (1994). Cadmium removal efficiency was reported to be 75–99.7%, lead removal was 26%, silver removal was 75.9%, and zinc removal was 66.7%. Sedimentation is a physical process after other mechanisms aggregate heavy metals into particles large enough to sink (Walker and Hurl, 2002).

(b) **Precipitation**: depends on the metal’s solubility product (Ksp) of the metal, pH of the wastewater, concentration of metal ions and relevant anions. When the values of the concentration of cations and anions are such that their product exceeds Ksp, precipitation takes place (Sheoran and Sheoran, 2006). Heavy metals in this way are removed from wastewater and trapped in the wetland sediments. Heavy metals in CWs may be adsorbed to soil or sediment, or may be chelated or complexed with organic matter. In addition to adsorption of heavy metals, oxide formation is also an important mechanism for metal removed from wastewater.

(c) **Biological** removal is also an important pathway for heavy metal removal in the CWs; it includes plant and microbial uptake. The rate of metal removal by plants varies widely, depending on plant growth rate, plant species and concentration of the heavy metals in the wastewater (Sheoran and Sheoran, 2006). Maximum concentration of metals in plants was observed in roots. Barley et al. (2005) also reported the highest metal concentrations in the roots of wetland plants (Barley et al., 2005).

(d) **Macrophytes** are known to accumulate a relatively high number of heavy metals in their biomass. These macrophytes are known as "hyperaccumulators" (Stottmeister et al., 2003). Heavy metal storage and uptake can also be measured in microorganisms. Microbial activity in CWs reduced metals to nonmobile forms, according to Sobolewski (1999).

- **Removal mechanism of Nutrients (Nitrogen and Phosphorus)**

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

Phosphorus: The fate of phosphorus in wetland soils is quite different, because there is no mechanism equivalent to de-nitrification because phosphorus does not have a gaseous phase. As a result, phosphorus tends to build in wetlands at a faster rate than nitrogen, despite the fact that plant uptake, sorption, decomposition, and long-term storage all occur. In wetlands with considerable storage or inputs of iron and aluminium (low pH wetlands) or calcium, precipitation of phosphate minerals can provide a significant sink for phosphorus (high-pH wetlands). While wetlands can take and store large amounts of phosphorus, they can also discharge a large quantity of phosphorus into downstream ecosystems. In a built wetland, the long-term removal rate of Phosphorus through plants is estimated to be around 0.05g/m2/day (Kapanen, 2008; Kim et.al, /2002).

MATERIALS AND METHODS

3.1 Pilot-scale constructed wetland (CW)

(a) (b)

s(c) (d)

Fig. 3.1 (a) and (b) *Cyperus alternifolius* before and after the experiment; (c) and (d) *Canna lily* before and after the experiment

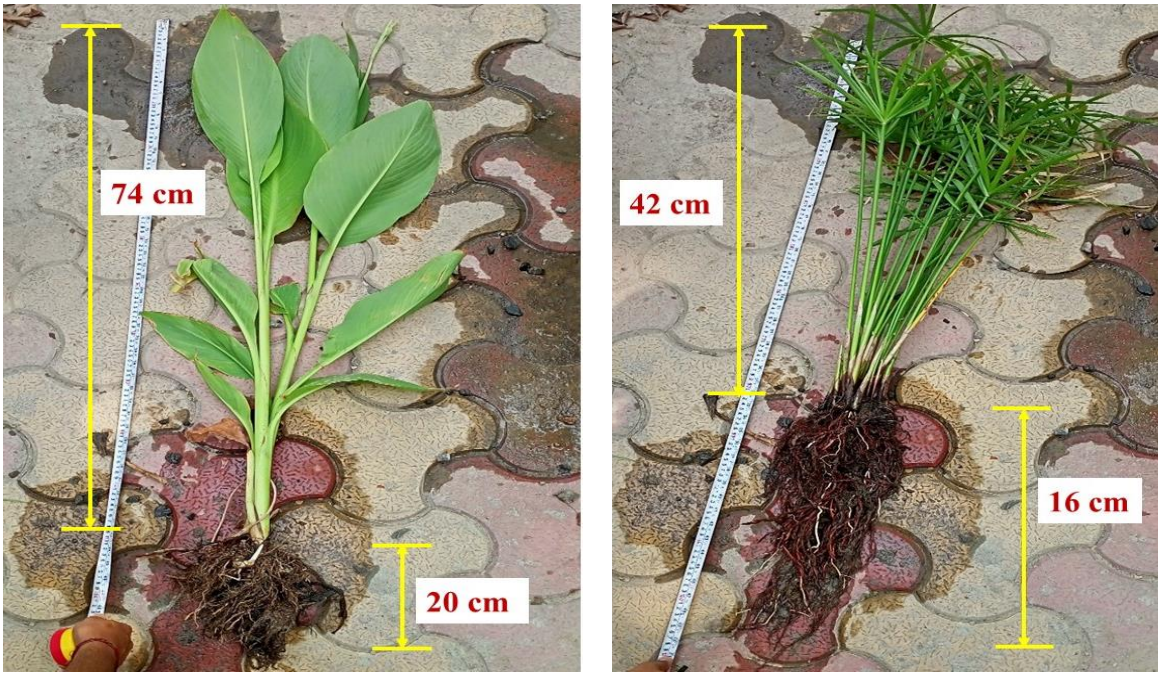
During the period between December, 2021 to May, 2022, the study was conducted in two pilot-scale vertical flow constructed wetlands (VFCW) in the campus of Delhi Technological University, New Delhi. To correlate the uptake of nutrients by *Canna* and *Cyperus*, prevailing weather conditions, the temperature profile during the period was examined together with other meteorological instances (*Annexure 1*). Both CW cells (*Canna lily* and *Cyperus alternifolius*) had a 38cm thick layer of sand-gravel substrate. Both cells were cylindrical tank constructed of high-density polyethylene (HDPE) plastic with a total height of 43 cm and an internal diameter of 55 cm. A free board of 5 cm was provided to retain the synthetic wastewater in CW cell. Thus, Water was kept at a height of 5 cm above the gravel surface. The effluent was collected from outlet tap provided at the bottom of the tank, and the influent was fed from the top having strength of 5 mg/l.

3.2 Adaptation of Plants

The CW cell was planted with 18 healthy *Canna lily* plants (stem count), with an average shoot length of 56.2 cm and an average root length of 10.5 cm. The plants had been planted previously for different purposes and were roughly 55 cm tall when the experiment began. In the case of *Cyperus alternifolius* CW cell, 12 plants with an average shoot length of 22 cm and an average root length of 11.5 cm were planted. Initially, time was given to the plants to stabilize. The CW cell was fed with urea and DAP as a source of nutrients before starting of the experimental work.

3.3 Selection of Plants

A considerable time was given to CW cell to get stabilized after which both the cell was flushed with distilled water initially, followed by flushing using type-1 water until the phosphate concentration in effluent becomes equal to concentration of type-1 water. At the end of the study, the total stem count and plant height for *Canna lily* were 21 and 82 cm, respectively, whereas the total stem count and height for *Cyperus alternifolius* were 27 and 20 cm, respectively.



(a) (b)

Fig. 3.2 After the experiment (a) Canna lily shoot length; (b) Cyperus alternifolius shoot length

During the experiment, the HRT (Hydraulic Retention Time) of wetlands was 24 hours. Both CW were fed 10 l/day of daily water with a phosphate concentration of 5 mg/l, which was synthetically prepared in the laboratory. The percentage of phosphate in waste water was found to vary between 3.4-6.8 mg/l.

3.4 Design of Experiments

(i) Preparation of Synthetic Wastewater

Analytical grade (AG) (Potassium Dihydrogen Orthophosphate and KH_2PO_4) salt was used to prepare the wastewater in the laboratory. Phosphate (PO_4^{3-}) was present in the effluent at consistent concentrations of 5 mg/l.

(ii) pH, EC and TDS

During the experiment, the pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured to assess the bed conditions. The pen type bench top pH metre used to measure pH, EC & TDS, Hanna instruments Italy; (Model: H19607).

(iii) Effect of Seasons

The primary objective was to study *Canna lily* and *Cyperus alternifolius* growth and phosphate removal effectiveness. The nutrient uptake capacity of both plants determines phosphate removal efficiency, and the uptake capacity of the plants is influenced by meteorological variables since greater daylight hours mean more photosynthesis, more metabolic processes, and thus more phosphate uptake from the wastewater. Different seasons, such as winter, spring, and summer were chosen to conduct the study. Rainfall (in mm) was also taken into account in the research as it can alter the influent synthetic wastewater strength through dilution.

3.5 Wastewater Analysis

For 5 months, effluent water samples were taken every 24 hours (HRT = 1 day). The fraction of phosphate concentration as available-phosphate (AP) and total-phosphate (TP) form was calculated using the standard method recommended by APHA (1997).

Phosphate-Fractions

Available Phosphate

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

Preparation of reagents

1) Ammonium Molybdate

25 gm of ammonium molybdate was dissolved in 175 ml of distilled water. In a separate conical flask add 280 ml of concentrated sulphuric acid in 400 ml of distilled water then mix the two solutions with intermittent mixing.

2) Stannous Chloride

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

3) Preparation of Standards

0.143 gm of potassium dihydrogen phosphate was mixed in 1 litre of distilled water to obtain 100 mg/l of phosphate stock standard. 10 ml of this solution was taken and final volume was made 100 ml to obtain 10 mg/l of phosphate standard. Serial distribution was used than to prepare 10 standards in the range of 0.1 to 1.0 mg/l.

Preparation of Standard curve

1) To 10 ml of 0.1 mg/l phosphate standard add 0.4 ml of ammonium molybdate and mix it well.

2) Add 2 drops of stannous chloride to it and mix it well.

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

4) Note the absorbance at 690 nm on spectrophotometer.

5) Repeat the above step for 0.2 mg/l, and upto 1.0 mg/l.

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

7) Absorbance shall be taken after 5 minutes after adding chemicals and before 11 minutes.

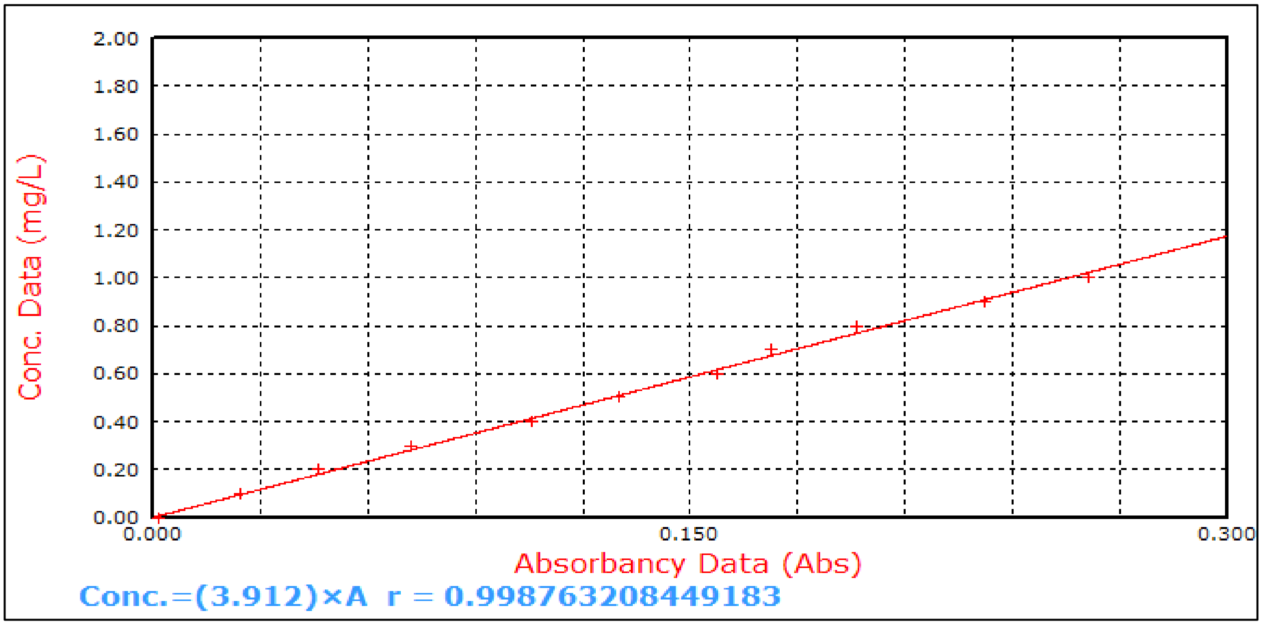


Fig. 3.3 Standard curve for phosphate (Labtronics make Model LT-290 Spectrophotometer)

Measurement of available phosphate

1) To 10 ml of sample, add 0.4 ml of ammonium molybdate and mix it well.

2) Add 2 drops of stannous chloride to it and mix it well.

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

4) Note the absorbance at 690 nm and 100% transmittance on spectrophotometer.

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

6) Absorbance shall be taken after 5 minutes after adding chemicals and before 11 minutes.

Total Phosphate

Total Phosphate is measured by Sulphuric Acid-Nitric acid digestion method.

Chemical Required

1) Concentrated Sulphuric acid (H_2SO_4)

2) Concentrated Nitric acid (HNO_3)

Apparatus Required

A microwave with at least 6 digestive units

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).

Measurement of Total Phosphate

1) To 10ml of sample adds 0.4 ml of conc.H_2SO_4 and to it add 2ml of conc. HNO_3.

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

3) After completion of digestion add NaOH solution to neutralize the solution. Neutralization can be seen as pink colour which appears after adding 2 drops of phenolphthalein before adding NaOH solution to the digested samples. Make up the volume to 50 ml using distilled water.

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

5) Add 2 drops of stannous chloride to it and mix it well.

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

7) Note the absorbance at 690 nm on spectrophotometer.

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

9) Absorbance shall be taken after 5 minutes after adding chemicals and before 11 minutes.

Measurement of Phosphate in Plant Tissue

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

Sample Preparation

1) Collect the various parts and left them for sun/ oven drying.

2) When enough drying was done then kept all the plant tissues in an oven for 1 day not more than 60° C.

3) Crush them by hand or mechanically and made a powdered form of these sample and collect them in separate container.

Measurement of Phosphate

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

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

3) After completion of digestion add NaOH solution to neutralize the solution. Neutralization can be seen as pink colour which appears after adding 2 drops of phenolphthalein before adding NaOH solution to the digested samples. Make up the volume to 50 ml using distilled water. To 50 ml of sample add 2 ml of ammonium molybdate and mix it well.

4) Add 2 drops of stannous chloride to it and mix it well.

5) Let it stand for 5 min till blue colour appears.

6) Note the absorbance at 690 nm on spectrophotometer.

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

RESULTS AND DISCUSSION

The purpose of this study is to monitor the removal efficacy of phosphate ions in *Canna lily* and *Cyperus alternifolius* over different seasons, as well as to investigate the effects of waste- water which was synthesized in the lab for both the plants. pH, electrical conductivity (EC), and total dissolved solids (TDS) were also evaluated on a regular basis during the study to identify any additional system alterations. Seasonal effects on removal behavior were also studied using meteorological data. The final results are listed below.

4.1 General Observations

The phosphate removal efficiency varies depending on the season, according to common findings. Phosphate removal efficiency was found to be lowest during the winter season and highest during the summer season in both CW cells. The total phosphate removal efficiency (%) of *Canna lily* CW cells is 77.6% < 82.6% < and 90.4% during the winter, spring, and summer seasons respectively. The same trend was seen for *Cyperus alternifolius* CW cell which also has the order of 62.4% < 74.2% < and 84.1% for winter, spring and summer respectively. Furthermore, the pH of the treated wastewater was almost in neutral range, indicating that both CW cells make the water more stable. Throughout the study, the electrical conductivity (EC) of the outlet from both CW cells was also enhanced. It climbed again once the experiment was restarted in the spring season, after covid-19 lockdown, and reaching upto 3160 µS/cm in the summer season. When compared to the winter season, EC increased for at both the inlet and outlet throughout the spring and summer seasons. This could be attributed towards the use of tap water during spring and summer season.

4.2 Ambient Temperature Profile

From the month of December 2021 to the month of May 2022, the ambient temperature profile was evaluated for about five months. The ambient temperature during the research ranged from 6 to 44 °C, with average maximum and minimum values of 10.3 °C and 40.7 °C, respectively. The minimum and maximum temperatures for the winter, spring, and summer seasons ranged from 6 to 26 °C; 12 to 40 °C; and 20 °C to 44 °C, respectively. The average ambient temperature in the winter, spring, and summer seasons was 15.9 °C, 25.7 °C, and 32.8 °C, respectively. The influence of ambient temperature on plants growth and phosphate removal efficiency was examined. The average number of hours of sunshine per day ranged between from 9 to 12 during the experiment. During the investigation, rainfall in millimeters (mm) was also measured. Rainfall occurred in short spurts (4 times), with the majority falling during the winter season (Annexure I). The intensity of rainfall that fell during these events ranged from 9.6 mm to 141.9 mm.

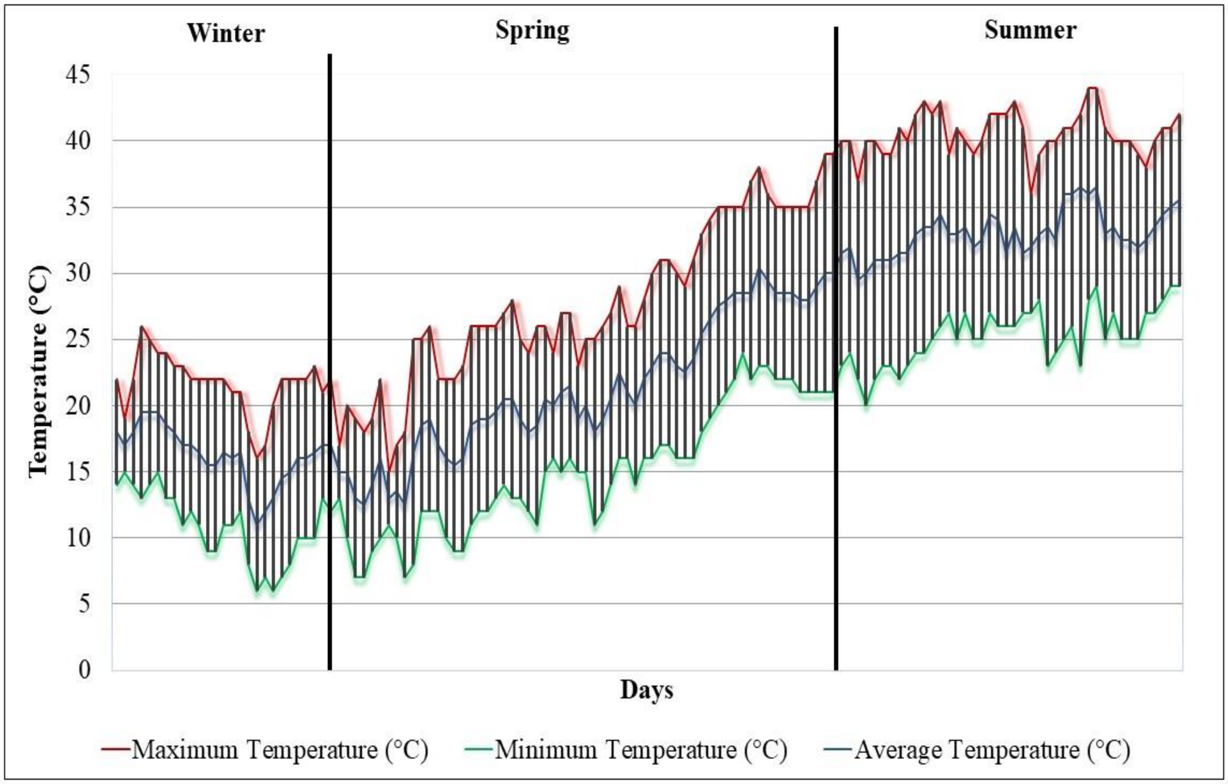


Fig. 4.1 Ambient temperature profile during the study (December, 2021 to May, 2022) Seasons classified as per IMD)

4.3 Sediment Sieve Analysis

The gap-graded gravel-sandy soil was packed into the beds of both the *Canna lily* CW cell and the *Cyperus alternifolius* CW cell. The packing material for the CW cell bed preparation has a specific gravity (G) of 2.6 with void (e) of 0.47, and bulk density of 16.82 kg/m^3. The CW cell has packing material constituted of 36.5% gravel (retained on a IS 4.75 mm sieve), 30% sand, and the rest was silt (passed through a IS 4.75 mm sieve throughout the analysis) (Table 4.1). Both CW cell beds are similar to the sort seen in natural wetlands. The voids in the gravels formed a porous medium that allowed for efficient and easy percolation of influent into the substrate and easy gas exchange at the root zone (rhizosphere). Gravels were used to form the bed so that the substrate would not clog. A gap graded soil with missing intermediate size particles is shown by the particle size distribution curve (Fig. 4.2). A gap graded soil has higher hydraulic conductivity and aids in the removal of phosphate from wastewater more effectively.

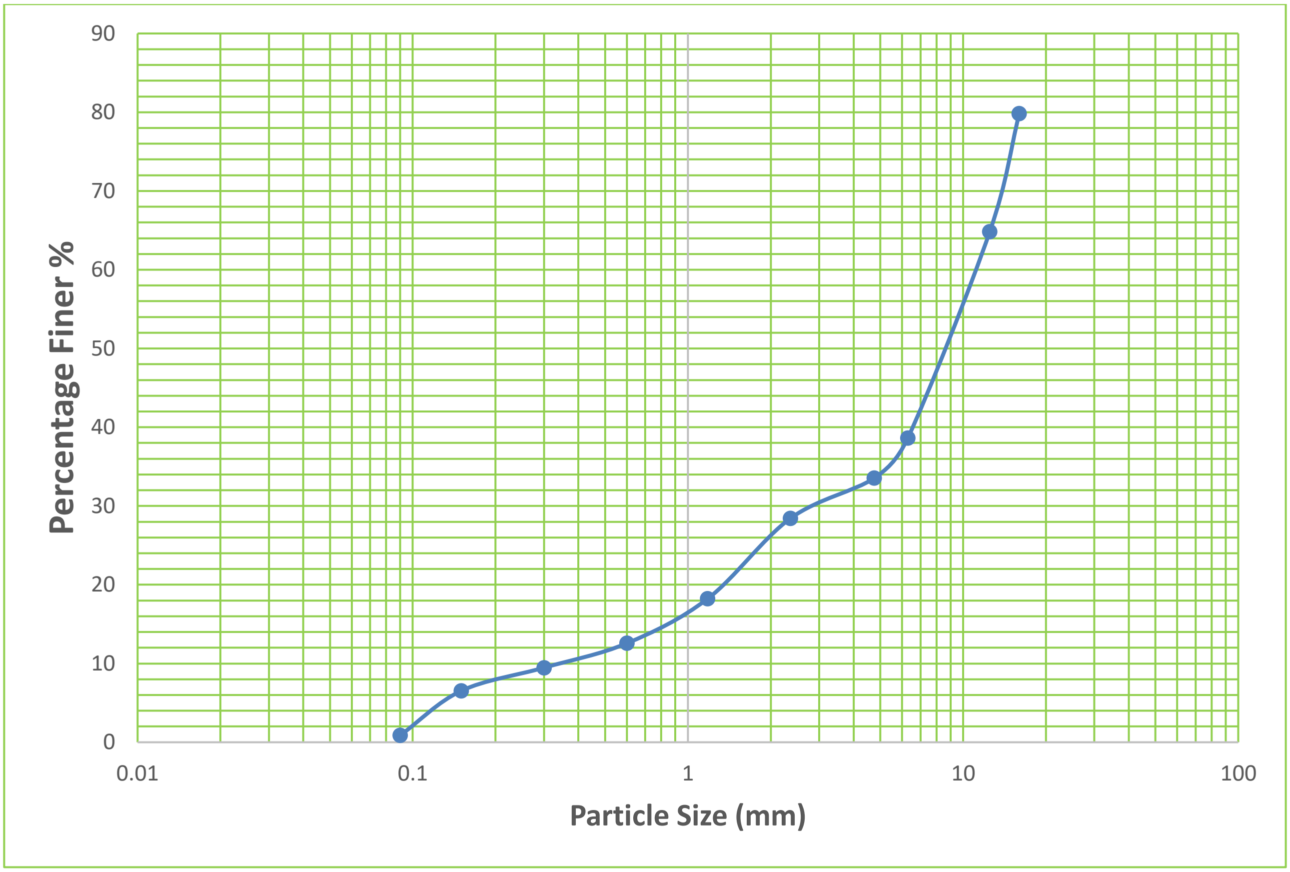


Figure 4.2 Sieve analysis and particle size distribution curve

Table 4.1 Sieve analysis of sediments

Sieve size (mm)	Weight retained (g)	Retained (%)	Cumulative (%)	% Finer
16	364	20.19	20.19	79.81
12.5	270	14.98	35.17	64.83
6.3	472	26.19	61.36	38.64

4.75	92	5.1	66.46	33.54
2.36	92	5.1	71.56	28.44
1.18	184	10.21	81.77	18.23
0.6	102	5.66	87.43	12.57
0.3	56	3.1	90.53	9,47
0.15	53	2.94	93.47	6.53
0.09	102	5.66	99.13	0.87
Pan	15	0.87	100	-
Total	1802	100	-	-

4.4 pH, EC and TDS of Wastewater

The pH of wastewater is an important measure to indicate its quality. Because of the addition of phosphate ions, the average pH of the influent (synthetic wastewater) during the study was in alkaline range (7.1 to 8.2). At a 5mg/l influent concentration, the effluent from the CW cell had an average pH of 7.6. The sediments and plants in wetland systems have been shown to remove enough dissolved nutrients and salts to make the water practically neutral. The CW cell functions as a buffering mechanism, maintaining the pH in neutral range regardless of pH variations, and can therefore absorb shocks in terms of influent pH. The average electrical conductivity (µS/cm) of influent increased from 412 to 1202 µS/cm. Since dissolved salts influence EC, a rise in TDS concentration was seen as the investigation continued. Average TDS increased from 210 mg/l to 598 mg/l. The dissolution of salts from the sediments in place of the nutrients removed from the wastewater may cause an increase in EC and TDS in effluent.

4.5 Nutrients Removal Study

Plants absorb nutrients from wastewater and use them in their metabolic processes. Depending on the environmental conditions, the chemical forms of nutrients contained in water change. Available phosphate (AP) was the more readily available to the plants among the two types of phosphate tested. The following table shows the impact of environmental variables (seasons) on the plant's growth and associated removal efficiency (%).

4.5.1 Seasonal Variations

Seasonal changes in the removal efficiency of different phosphate fractions were studied from December 2021 to May 2022. Winter, spring, and summer were the seasons studied in chronological order during the study. The % removal efficiency of phosphate was studied for both available and total phosphates. The wastewater was synthetically prepared for inlet, and the treated water was collected and analysed from the CW cells after a 24-hour hydraulic retention time (HRT). Both the inlet and outlet are analyzed for TP and TP.

Winter season

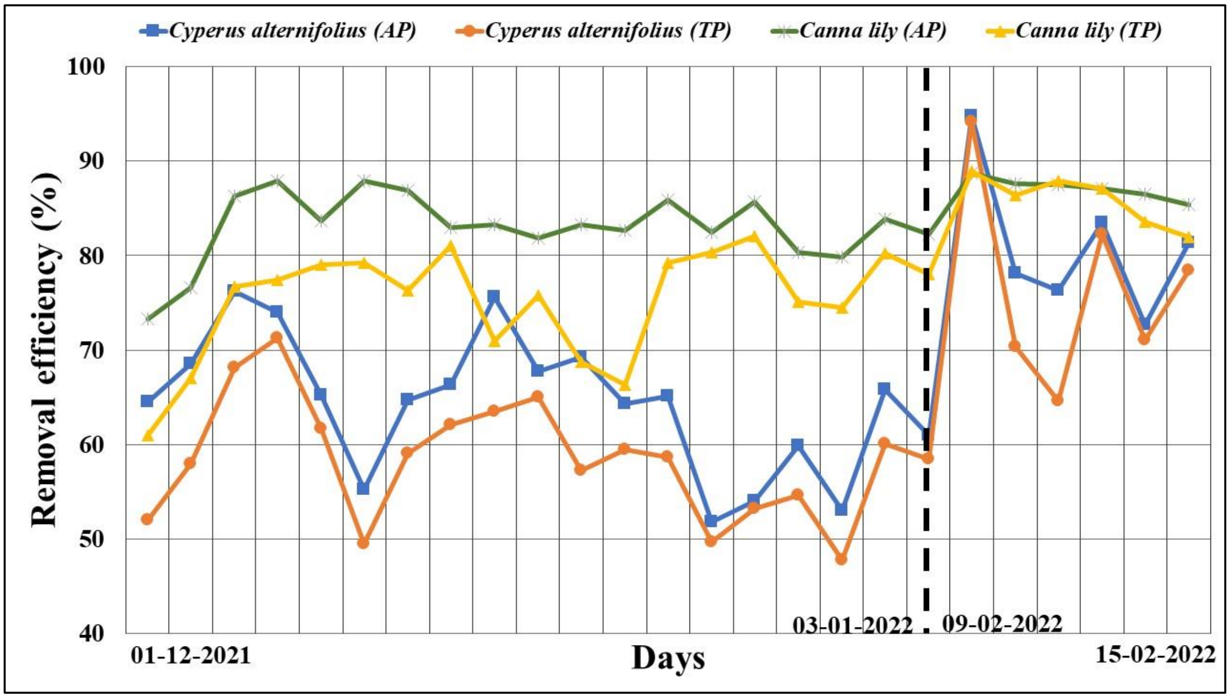


Fig. 4.3 Removal efficiency (%) of available and total phosphate in effluent from *Canna lily* and *Cyperus alternifolius* based CW Cell during winter season.

The influence of temperature change on phosphate removal efficiency and the growth of both *Canna lily* and *Cyperus alternifolius* was studied over winter season from December 2021 to February 2022. The average initial concentration of phosphate (PO_4^{3-}-P) fed to both *Canna lily* and *Cyperus alternifolius* CW cells was 5.2 ±SD mg/l over the study period, which varied from 4.0 mg/l to 6.5 mg/l. (Table 4.3). The fraction of AP in the inlet of both *Canna lily* and *Cyperus alternifolius* varied from 4.2 mg/l to 5.9 mg/l, with an average of 4.9 ±SD mg/l. The AP concentration in the *Canna lily* outlet varied from 0.5 to 1.3 mg/l, with an average of 0.7 ±SD mg/l. The average AP removal efficiency was 83.7 %, ranging between 73.3% to 88.8%. The average removal efficiency of TP was 77.5 %, which was lower than that of AP removal efficiency (83.7 %), and it ranged from 60 % to 88.9 %.

The proportion of AP in the outflow of the *Cyprus Alternifolius* CW cell ranged from 1.0 mg/l to 2.4 mg/l, with an average of 1.5 ±SD mg/l. The average AP removal efficiency was 67.43 %, which varied between 51.9% and 94.8%. TP average removal efficiency was 62.3% which is less than AP (67.4%), and it ranged from 47.3% to 94.3%.

The most probable reason is that unbound accessible phosphate (AP) is more conveniently available to plants and is taken up by the plant for its metabolic activities. In addition, as compared to the pre-lockdown period, the average removal efficiency was significantly high during the winter season after the lockdown. The improved removal efficiency after the lockdown phase can be attributed towards the higher hydraulic retention time (HRT) during which plants performed their metabolic activities, and thus, shown significantly high removal efficiency.

Table 4.2 AP and TP concentration and its removal efficiency (%) during winter seasons for PO_4^{3-}-P = 5mg/l

Date (n=25)	**Inlet**		**Outlet *Cyprus alternifolius***				**Outlet *Canna lily***			
	AP (mg/l)	**TP (mg/l)**	**AP (mg/l)**	**TP (mg/l)**	**AP Removal efficiency (%)**	**TP Removal efficiency (%)**	**AP (mg/l)**	**TP (mg/l)**	**AP Removal efficiency (%)**	**TP Removal efficiency (%)**
30-11-2021	4.9	5.5	1.7	2.6	**64.4**	**52.0**	1.3	2.1	**73.3**	**60.9**
01-12-2021	4.81	5.2	1.5	2.2	**68.6**	**57.9**	1.1	1.7	**76.5**	**66.9**
02-12-2021	4.8	5.2	1.1	1.6	**76.2**	**68.1**	0.6	1.2	**86.3**	**76.6**
03-12-2021	5.5	5.8	1.4	1.6	**73.9**	**71.3**	0.6	1.3	**87.9**	**77.4**
06-12-2021	5.3	5.9	1.8	2.2	**65.1**	**61.6**	0.8	1.2	**83.6**	**78.9**
08-12-2021	4.1	4.5	1.8	2.3	**55.2**	**49.4**	0.5	0.9	**87.9**	**79.2**
09-12-2021	4.9	5.0	1.7	2.0	**64.7**	**59.0**	0.6	1.2	**86.8**	**76.3**
10-12-2021	5.1	5.6	1.7	2.1	**66.2**	**62.1**	0.8	1.0	**82.9**	**81.0**
13-12-2021	4.6	4.0	1.1	1.4	**75.6**	**63.5**	0.7	1.1	**83.2**	**71.0**
15-12-2021	5.2	5.5	1.7	2.0	**67.7**	**64.9**	0.9	1.3	**81.8**	**75.7**
16-12-2021	4.8	5.0	1.5	2.2	**69.2**	**57.3**	0.8	1.5	**83.2**	**68.7**
17-12-2021	4.9	5.0	1.8	2.0	**64.3**	**59.4**	0.8	1.7	**82.6**	**66.3**
20-12-2021	5.0	5.2	1.8	2.2	**65.1**	**58.6**	0.7	1.0	**85.8**	**79.2**
22-12-2021	4.7	5.4	2.3	2.7	**51.7**	**49.6**	0.8	1.0	**82.4**	**80.3**

23-12-2021	5.0	5.2	2.3	2.5	**54.0**	**53.2**	0.7	0.9	**85.7**	**82.1**
24-12-2021	4.5	4.7	1.8	2.1	**59.8**	**54.6**	0.9	1.2	**80.3**	**75.1**
27-12-2021	4.2	4.4	2.0	2.3	**53.0**	**47.7**	0.9	1.1	**79.8**	**74.5**
29-12-2021	5.3	5.7	1.8	2.3	**65.8**	**60.0**	0.9	1.1	**83.9**	**80.2**
30-12-2021	5.2	5.8	2.0	2.4	**60.9**	**58.4**	0.9	1.2	**82.2**	**78.0**
03-01-2022	5.2	5.6	0.3	0.3	**94.8**	**94.1**	0.6	0.6	**88.7**	**88.9**
09-02-2022	4.8	5.2	1.0	1.5	**78.1**	**70.4**	0.6	0.7	**87.6**	**86.4**
10-02-2022	4.8	5.2	1.2	1.8	**76.2**	**64.5**	0.6	0.6	**87.5**	**87.9**
11-02-2022	5.2	5.6	0.9	1.0	**83.4**	**82.2**	0.6	0.7	**87.1**	**87.1**
14-02-2022	5.9	6.5	1.6	1.9	**72.6**	**71.0**	0.8	1.0	**86.5**	**83.5**
15-02-2022	5.4	5.6	1.0	1.2	**81.3**	**78.4**	0.7	1.0	**85.3**	**81.9**
Min.-Max.	5.9 - 4.2	6.5 - 4.0	2.4 - 1.0	2.6 - 0.3	**94.8 - 51.9**	**94.1 - 47.7**	1.3 - 0.5	2.1 - 0.6	**88.7 - 73.3**	**88.9 - 60.9**
Mean ± SD	4.9 ± 0.3	5.2 ± 0.6	1.6 ± 0.4	1.9 ± 0.5	**67.4 ± 10.0**	**62.3 ± 10.4**	0.7 ± 0.1	1.1 ± 0.3	**83.7 ± 3.4**	**77.5 ± 6.4**

**AP = Available phosphate; *TP = Total phosphate; *SD= Standard deviation*

Spring season

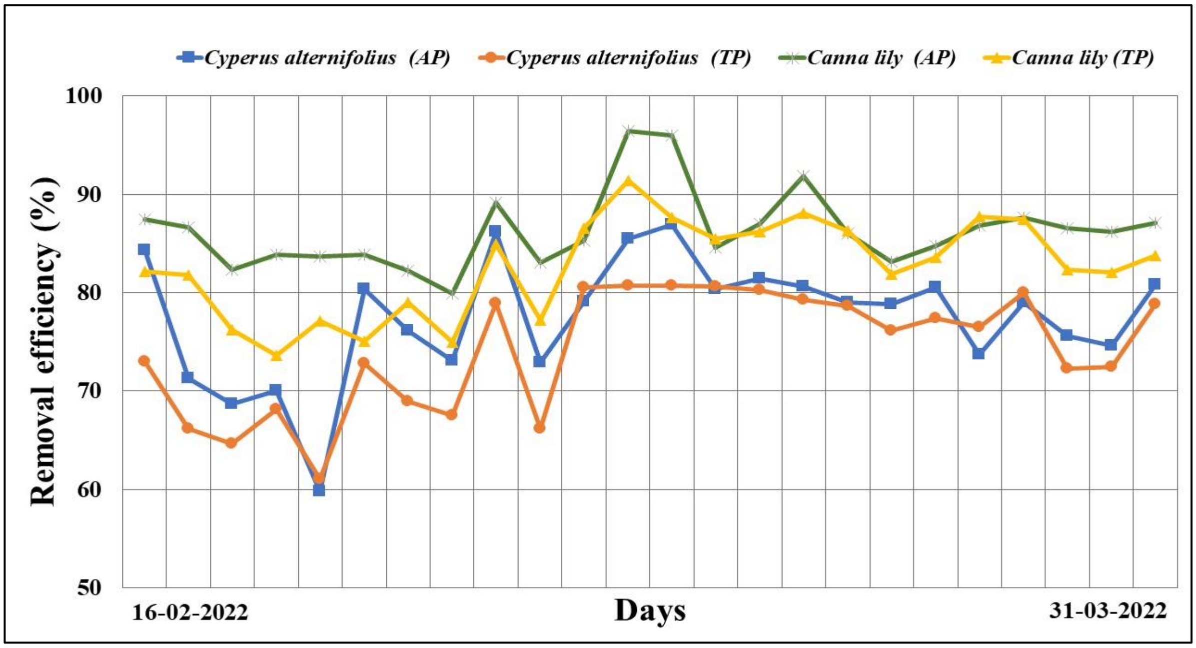


Fig. 4.4 Removal efficiency (%) of available and total phosphate in effluent from *Canna lily* and *Cyperus alternifolius* based CW Cell during spring season.

The spring season is described as the time period from mid-February to March in 2022. During the spring, it was found that plants require nitrogen as an external input or they will undergo stress. As a result, diammonium phosphate (DAP) was introduced to CW cells during the spring season. The addition of DAP to the wastewater treatment process enhanced phosphate removal. The average inlet phosphate concentration was 4.8 ± SD mg/l, which ranges from 3.3 mg/l to 5.6 mg/l. The abundance of nutrients in the CW cell may be responsible for the improvement in removal efficiency. The AP outlet concentrations for *Canna lily* ranged from 0.2 mg/l to 0.8 mg/l, with an average of 0.6 ±SD mg/l, with an 86.3 % removal efficiency, which is greater than TP. Same set of observation has been observed for *Cyperus alternifolius* with an average removal efficiency of 77.4%. Another factor for the removal efficiency during the spring season could be the progressive increase in ambient temperature and average daylight hours. During that time, the average daily temperature temperature rised from 16 to 25.5°C. Enhanced evapotranspiration and metabolic activity causes an increased updraft of nutrient from the roots to the leaves of plants when the temperature and sunshine hours rises.

Table 4.3 AP and TP concentration and its removal efficiency (%) during spring seasons for PO_4^{3-}-P = 5mg/l

Date (n=25)	**Inlet**		**Outlet *Cyprus alternifolius***				**Outlet *Canna lily***			
	AP (mg/l)	**TP (mg/l)**	**AP (mg/l)**	**TP (mg/l)**	**AP Removal efficiency (%)**	**TP Removal efficiency (%)**	**AP (mg/l)**	**TP (mg/l)**	**AP Removal efficiency (%)**	**TP Removal efficiency (%)**
17-02-2022	4.4	4.7	0.7	1.2	**84.3**	**73.0**	0.5	0.8	**87.4**	**82.1**
18-02-2022	4.6	4.0	1.3	1.3	**71.2**	**66.1**	0.6	0.7	**86.6**	**81.7**
22-02-2022	4.7	5.3	1.5	1.8	**68.6**	**64.6**	0.8	1.2	**82.3**	**76.2**
23-02-2022	4.8	5.5	1.4	1.7	**69.9**	**68.1**	0.7	1.4	**83.8**	**73.6**
24-02-2022	4.3	5.0	1.7	1.9	**59.7**	**61.0**	0.7	1.1	**83.7**	**77.1**
25-02-2022	4.8	5.4	0.9	1.4	**80.3**	**72.8**	0.7	1.3	**83.8**	**75.0**
03-03-2022	4.6	4.9	1.1	1.5	**76.1**	**68.9**	0.8	1.0	**82.3**	**78.0**
04-03-2022	3.3	3.7	0.8	1.2	**73.0**	**67.5**	0.6	0.9	**79.9**	**75.0**
05-03-2022	5.8	6.2	0.8	1.3	**86.1**	**78.9**	0.6	0.9	**89.1**	**84.9**
07-03-2022	4.3	4.9	1.1	1.7	**72.9**	**66.1**	0.7	1.1	**83.0**	**77.2**
08-03-2022	4.7	5.2	1.0	1.0	**79.1**	**80.5**	0.7	0.8	**85.3**	**86.5**
09-03-2022	5.6	6.0	0.8	1.1	**85.5**	**80.7**	0.2	0.5	**96.4**	**91.4**
10-03-2022	5.6	6.2	0.7	1.2	**86.9**	**80.7**	0.2	0.7	**95.0**	**87.7**
11-03-2022	4.3	4.9	0.8	0.9	**80.4**	**80.6**	0.7	0.7	**84.5**	**85.4**

14-03-2022	4.9	5.6	0.9	1.1	**81.4**	**80.2**	0.6	0.7	**87.0**	**86.2**
15-03-2022	5.0	5.5	0.9	1.1	**80.7**	**79.3**	0.4	0.6	**91.8**	**88.1**
16-03-2022	5.1	6.0	1.0	1.3	**79.0**	**78.7**	0.7	0.8	**86.0**	**86.3**
21-03-2022	4.2	4.5	0.9	1.1	**78.8**	**76.1**	0.7	0.8	**83.1**	**81.9**
23-03-2022	4.9	5.8	0.9	1.3	**80.5**	**77.4**	0.7	0.9	**84.8**	**83.6**
24-03-2022	4.5	5.3	1.1	1.2	**73.7**	**76.5**	0.6	0.6	**86.9**	**87.7**
25-03-2022	4.5	5.0	0.9	1.0	**79.0**	**79.9**	0.5	0.6	**87.6**	**87.5**
28-03-2022	5.2	5.3	1.2	1.5	**75.6**	**72.3**	0.7	0.9	**86.6**	**82.3**
29-03-2022	4.2	4.9	1.0	1.4	**74.6**	**72.4**	0.6	0.8	**86.2**	**82.0**
31-03-2022	5.0	5.5	0.9	1.1	**80.8**	**78.8**	0.6	0.9	**87.1**	**83.7**
Min.-Max.	5.6-3.3	6.2-3.7	1.7-0.7	1.2-0.9	**86.9-59.8**	**80.7-61.0**	0.8-0.2	1.6-0.5	**96.4-82.3**	**91.4-73.6**
Mean	4.8	5.2	1.0	1.3	**77.4**	**74.2**	0.6	0.9	**86.3**	**82.6**
± SD	± 0.5	± 0.6	± 0.2	± 0.2	**± 6.2**	**± 6.1**	± 0.1	± 0.2	**± 3.4**	**± 4.9**

**AP = Available phosphate; *TP = Total phosphate; *SD= Standard deviation*

Summer Season

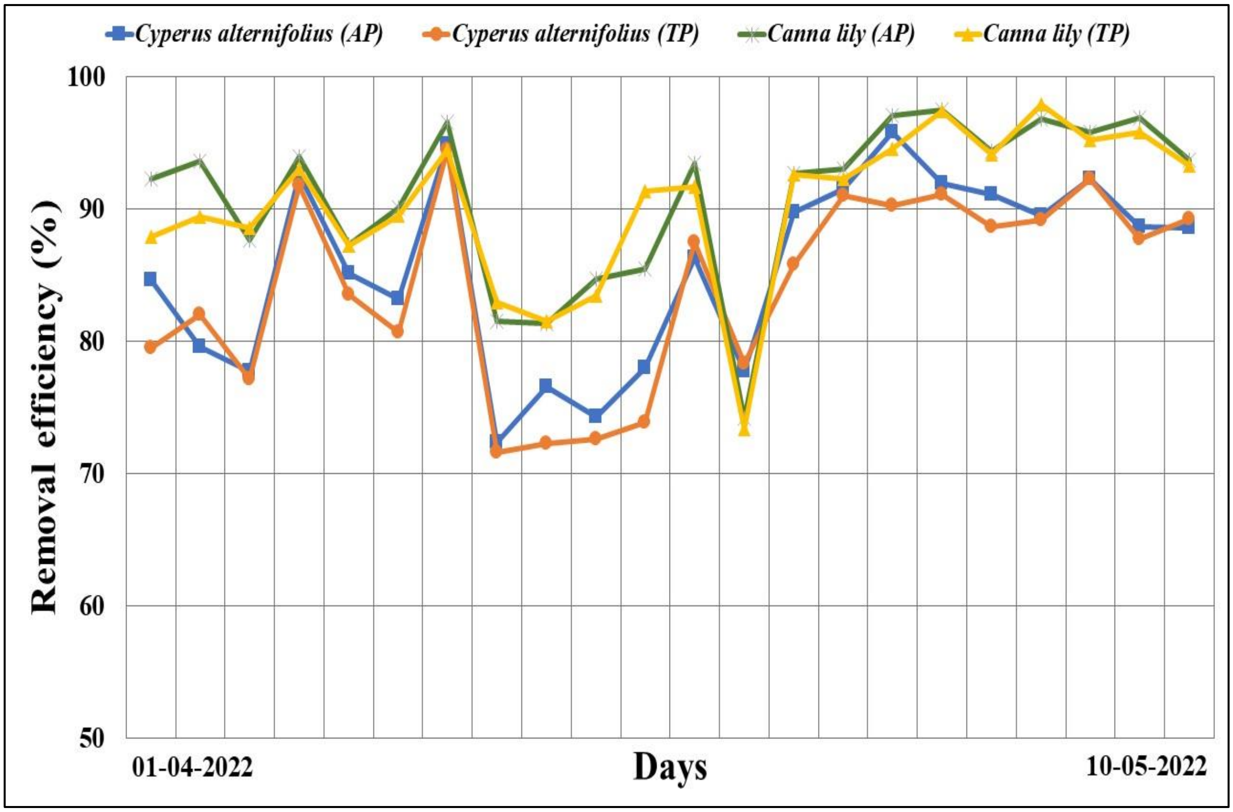


Fig. 4.5 Removal efficiency (%) of available and total phosphate in effluent from *Canna lily* and *Cyperus alternifolius* based CW Cell during summer season.

During the study, the summer season was described as the time from the first week of April 2002 to the first week of May 2022. External nutrients were not added to the inlet during this time. The inlet total phosphate inlet concentrations averaged 5.9 ± SD mg/l, varying from 5.2 mg/l to 7.0 mg/l (Table 4.4). The concentration of TP in the outlet of *Canna lily* ranged from 0.1 to 1.8 mg/l, with an average of 0.5 ± SD mg/l and a removal efficiency of 90.4 %. In contrast, the average AP concentration in effluent was 0.4 ± SD mg/l, compared to 5 mg/l in the influent. The removal efficiency varied from 74.2 % to 97.6 %, with an average of 91 %, which was significantly higher than the winter and spring seasons. The TP concentration in *Cyperus alternifolius* ranged from 0.3 mg/l to 1.5 mg/l on average, with a removal efficiency of 84 %. AP, on the other hand AP concentration, had an average outlet concentration of 0.7 mg/l. It has a removal efficiency that ranges from 73% to 95.5 %, with an average of 86 %. In the summer, first-order kinetics represents an improvement in removal efficiency with an increase in phosphate concentration. The simple bioavailability of AP to plants and microorganisms, as opposed to the limited stock of TP, could explain the higher removal efficiency for AP. The pH of water and sediments in CW cells, as well as the availability of metals such as Ca, Mg, Al, and Fe in sediments and wastewater, determine whether TP is available to wetland vegetation because it could only be available after its bioconversion to AP.

Table 4.4 AP and TP concentration and its removal efficiency (%) during summer season for PO_4^{3-}-P = 5mg/l

Date (n=20)	**Inlet**		**Outlet *Cyperus alternifolius***				**Outlet *Canna lily***			
	AP (mg/l)	**TP (mg/l)**	**AP (mg/l)**	**TP (mg/l)**	**AP Removal efficiency (%)**	**TP Removal efficiency (%)**	**AP (mg/l)**	**TP (mg/l)**	**AP Removal efficiency (%)**	**TP Removal efficiency (%)**
01 April 2022	4.7	5.2	0.7	1.1	**84.6**	**79.5**	0.4	0.6	**92.3**	**87.9**
06 April 2022	5.6	5.9	1.1	1.1	**79.5**	**82.0**	0.3	0.6	**93.6**	**89.4**
07 April 2022	5.2	5.8	1.1	1.3	**77.7**	**77.1**	0.6	0.7	**87.6**	**88.6**
08 April 2022	6.0	6.0	0.4	0.5	**92.6**	**91.8**	0.3	0.4	**94.0**	**93.0**
11 April 2022	5.6	6.2	0.8	1.0	**85.1**	**83.5**	0.7	0.8	**87.3**	**87.2**
12 April 2022	5.4	5.9	0.9	1.1	**83.1**	**80.6**	0.5	0.6	**90.0**	**89.4**
14 April 2022	5.3	6.0	0.2	0.3	**94.8**	**94.5**	0.2	0.3	**96.6**	**94.5**
18 April 2022	4.8	5.4	1.3	1.5	**72.3**	**71.6**	0.9	0.9	**81.5**	**82.9**
19 April 2022	4.8	5.4	1.1	1.5	**76.5**	**72.2**	0.9	1.0	**81.4**	**81.5**
20 April 2022	5.0	5.4	1.3	1.5	**74.3**	**72.6**	0.7	0.9	**84.7**	**83.5**
21 April 2022	5.0	5.5	1.1	1.4	**77.9**	**73.9**	0.7	0.4	**85.5**	**91.4**
22 April 2022	5.0	5.6	0.7	0.7	**86.2**	**87.4**	0.3	0.4	**93.4**	**91.7**
25 April 2022	6.0	6.8	1.3	1.5	**77.7**	**78.3**	1.6	1.8	**74.21**	**73.3**
26 April 2022	5.5	6.2	0.6	0.9	**89.7**	**85.8**	0.4	0.4	**92.7**	**92.6**

27 April 2022	6.1	6.4	0.5	0.6	**91.5**	**91.0**	0.4	0.5	**93.0**	**92.3**
29 April 2022	5.9	6.0	0.2	0.6	**95.8**	**90.2**	0.2	0.3	**97.1**	**94.6**
02 May 2022	6.3	7.0	0.5	0.6	**91.9**	**91.1**	0.1	0.2	**97.5**	**97.4**
04 May 2022	4.9	5.5	0.4	0.6	**91.1**	**88.6**	0.3	0.3	**94.3**	**94.1**
05 May 2022	6.0	6.3	0.6	0.7	**89.5**	**89.1**	0.2	0.1	**96.7**	**97.9**
06 May 2022	6.3	6.8	0.5	0.5	**92.3**	**92.3**	0.2	0.3	**95.8**	**95.2**
09 May 2022	5.5	6.3	0.6	0.8	**88.7**	**87.7**	0.1	0.2	**96.9**	**95.8**
10 May 2022	5.3	5.9	0.6	0.6	**88.6**	**89.2**	0.3	0.4	**93.7**	**93.3**
Min.-Max.	6.3-4.8	6.8-5.2	1.3-0.2	1.5-0.3	**95.8-72.4**	**91.9-71.6**	1.5-0.6	1.8-0.2	**97.5-74.2**	**97.4-73.3**
Mean	5.4	5.9	0.7	0.9	**85.5**	**84.1**	0.4	0.5	**90.9**	**90.3**
± SD	± 0.5	± 0.4	± 0.3	± 0.3	**± 7.0**	**± 7.3**	± 0.3	± 0.3	**± 6.2**	**± 5.9**

**AP = Available phosphate; *TP = Total phosphate*; **SD= Standard deviation*

4.6 Combined % removal efficiency for different seasons

Because the extraction of phosphate (AP and TP) by both *Canna lily* and *Cyperus alternifolius* is mostly influenced by environmental conditions, different seasons have distinct effects on plant removal behavior. Temperature has a considerable impact on the rate at which nutrients are removed. The rate of intake of water and nutrients from an aquatic system was found to be closely proportional to temperature, as the higher the evapotranspiration, the greater the rate of uptake of water and nutrients.

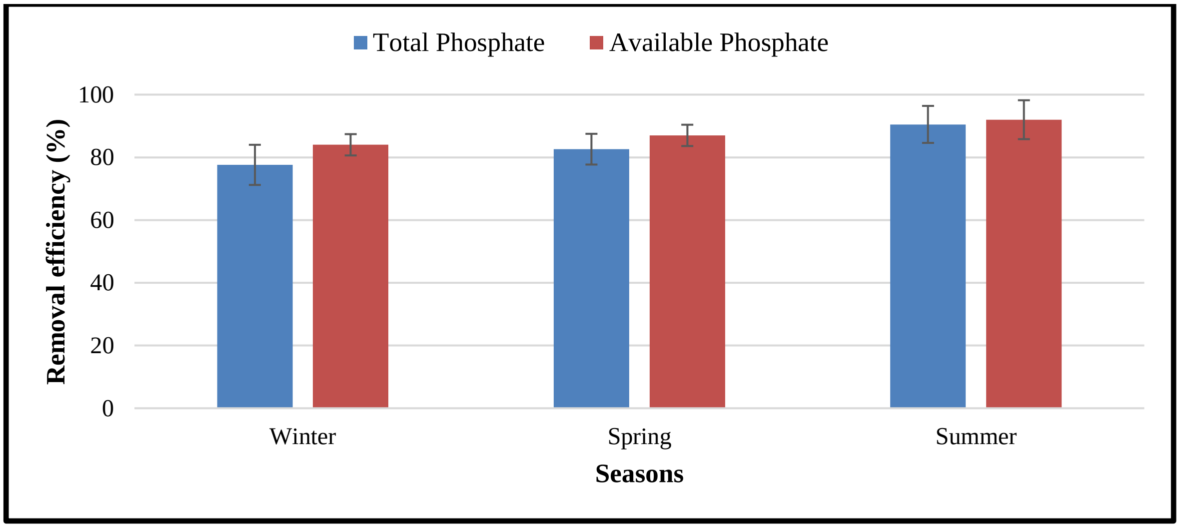


Fig. 4.6 Variation in average phosphate removal efficiency (%) of *Canna lily* for different seasons.

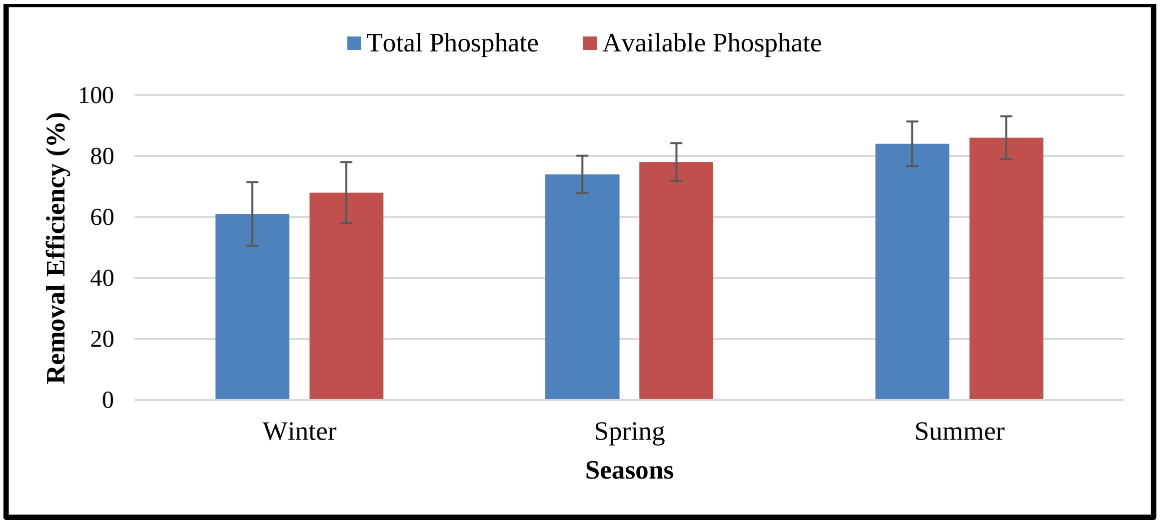


Fig. 4.7 Variation in average phosphate removal efficiency (%) of *Cyperus alternifolius* for different seasons.

With the exception of a substantial drop in the late winter season, similar results were observed in the current study (Fig 4.6 and Fig. 4.7). The cause could be due to nutritional stress in the plants. In a wetland system, it is consequently necessary to maintain the appropriate supply of nutrients (CNP). The removal effectiveness of AP was quite higher than that of TP, and it improved as the seasons progressed from winter to summer for both plants. (Fig. 4.6 and Fig. 4.7).

4.7 Analysis of Plant Tissue

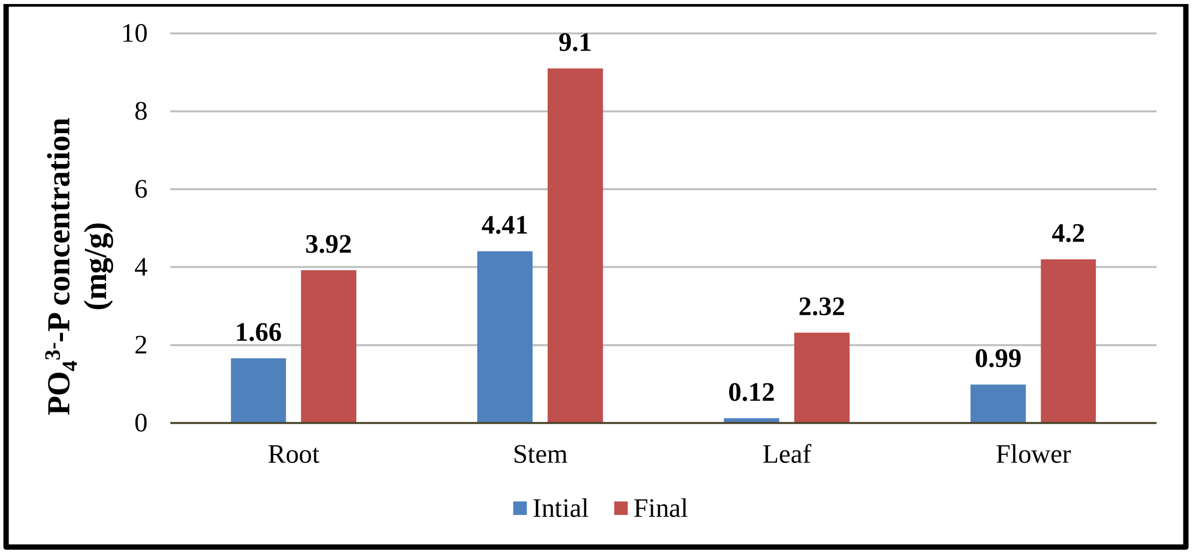


Fig 4.8 Total Phosphate concentration (mg/g) in different plant tissues for *Canna lily*

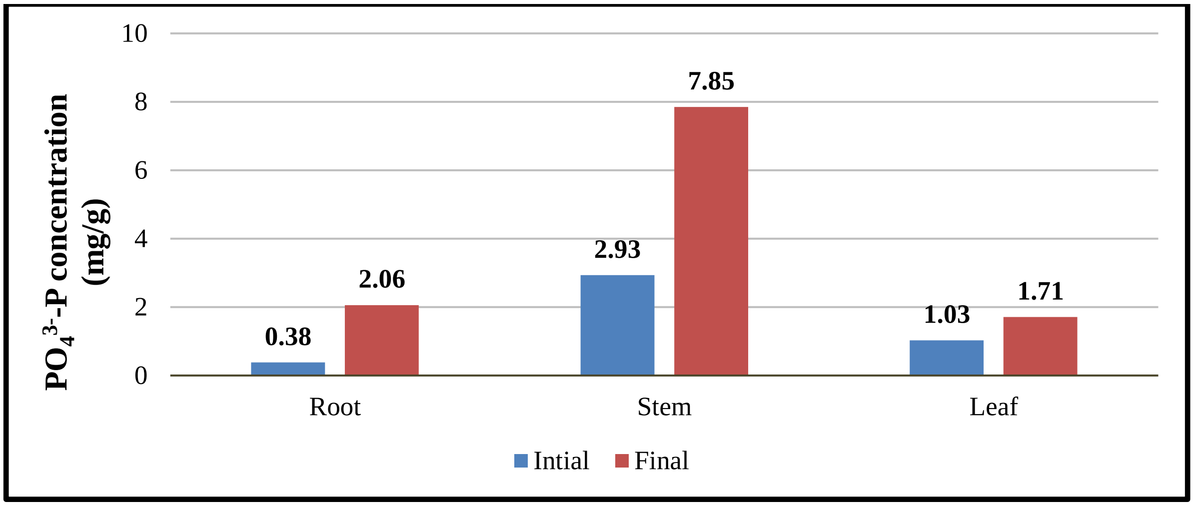


Fig 4.9 Total Phosphate concentration (mg/g) in different plant tissues for *Cyperus alternifolius*

Considering phosphate removal efficiency is dependent on the plant, or more precisely, the its phosphate uptake efficiency. It is crucial to understand phosphorus accumulation in various parts of plant tissue. Two plant samples were taken from each plant in this study. Initial plant sample collected before the start of experiment and a final plant sample collected after the experiment was completed. The amount of phosphate accumulated in various portions of the plant was evaluated in order to estimate translocation of phosphate in parts of the plant by uptake from waste water. The accumulation of phosphate in various parts of the initial and final samples is shown in Fig 4.8 and Fig. 4.9 for both plants. The highest phosphorus concentration was detected in the stem and the lowest concentration was found in the roots in both the initial and final samples for *C. alternifolius*. Whereas, in case of *C. lily*, the highest phosphorus concentration was found in the stem and the lowest was in flower. It could mean that the phosphate is plants taken up by the plant is utilised for their metabolic activity.

4.8 Effect of wastewater on plants

Since nutrients like phosphate are required for plant growth and metabolism, it is crucial to assess the growth of plant during the study period. Plant growth was measured in terms of average root length, average shoot length, shoot: root ratio, and average plant density in this study.

The rise in root length, shoot length, and plant density can be seen in Tables 4.5. This means that neither plant will be stressed by the waste water, and their growth will be normal. *Canna lily* and *Cyperus alternifolius* species planted in the wetland grew well in this study, and the number of plants per unit area also increased. This reveals that waste water containing phosphate at concentrations ranging from 3 to 7 ppm does not inhibit but rather encourages photosynthetic and metabolic activity. This implies that phosphate in waste water has a favorable effect on both plant growth and reproduction. The interaction of plants, microorganisms, and wastewater may be responsible for the removal of phosphate from wastewater. For their growth and metabolism, plants have taken up phosphate from wastewater. Microbes in the soil release a variety of enzymes that convert various types of phosphate into usable forms. It will aid in both plant development and the elimination of phosphate from waste water.

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

Parameter	***Canna lily***		***Cyperus alternifolius***	
	Initial	**Final**	**Initial**	**Final**
Shoot length (cm)	56	94	14	42
Root length (cm)	10.5	20.5	4.5	16
Shoot: Root Ratio	5.33	4.59	3.11	2.62
No. of stems	8	21	10	62
Plant Density (unit/m^2)	16	42	20	124

CONCLUSION

The following conclusions were drawn based on the findings of this experiment.

1. *Canna lily* and *Cyperus alternifolius* are both able to thrive in the wetland environments of tropical India. It worth noting that the influent should be high in nitrogenous materials, as a nitrogen deficit combined with low temperatures might cause plant stress, as seen in this study.

2. *Canna lily* and *Cyperus alternifolius* can both flourish in semi-arid climates with temperatures ranging from 6 to 45 °C. It has an average phosphate removal efficiency of 77% to 90% for *Canna lily* and for *Cyperus alternifolius* from 65% to 80% wastewater in Indian circumstances.

3. CW cells based on *C. lily* and *C. alternifolius* successfully extract phosphate, however there may be an increase in TDS in the effluent after treatment. As a result, the dissolution of different chemical species from the CW cell bed is concerning, and the regulating factors may be addressed.

4. Both plant species shown removal behaviour which is strongly influenced by meteorological parameters, particularly temperature and day light hours. The rate of evapotranspiration is regulated by temperature; the higher the temperature, the greater the rate of evapotranspiration, resulting in a greater demand of water and nutrients.

5. The Total Phosphate concentration (mg/g) in different tissues of plant increased and revealed that it plays an important role in growth and metabolisms of plants. The highest phosphorus concentration was detected in the stem and the lowest concentration was found in the roots in both the initial and final samples for *C. alternifolius*. Whereas, in case of *C. lily*, the highest phosphorus concentration was found in the stem and the lowest was in flower.

6. Both plants are a promising tool in phosphate removal from waste water without any stress of influent concentration (phosphate) and temperature fluctuations. The plant can be used in stabilization of waste water under remain conditions.

SUMMARY

A wetland is an area of land where the soil is constantly or seasonally saturated with moisture, giving it the features of a unique ecosystem. The ability of both natural and manmade wetlands to purify wastewater is widely recognized in western world & limited studies are based in India. Pollutants are removed from wetlands by a complicated system of biological, physical, and chemical processes. There are numerous benefits to employing a built wetland system over a traditional system. The disposal of secondary pollutants (such as sludge) is a major issue in conventional treatment systems, whereas, there is no such issue in polluted removal from wetlands. The goal of using a constructed wetland (CW) as an advanced treatment is to remove nutrients (phosphorus and nitrogen compounds) that are disruptive to the balance of more sensitive receiving media, causing eutrophication. Wetlands are less expensive to develop than any other treatment method. Because CW is a natural process that does not require continuous monitoring, its operation and maintenance costs (energy and supplies) are minimal. Different water-tolerant plant species are used in wetlands treatment systems. Surface flow (SF) wetlands and subsurface flow (SSF) wetlands are the two most common forms of manmade wetland treatment systems. Wetland vegetation is divided into three categories: 1) emergent aquatic macrophytes (such as *Typha*, *Canna lily*, and *Cyperus alternifolius*), 2) floating-leaved aquatic macrophytes (such as Water hyacinth, Duckweed), and 3) submerged aquatic macrophytes eg. Hydrilla. The majority of constructed wetlands research has been undertaken in European countries. As a result, the plants that have been examined the most for treatment purposes are those that can withstand freezing temperatures. However, not much studies were undertaken to study the effectiveness of attractive plants in treatment wetlands in India. This study looked into the application of ornamental plants in treatment wetlands in India. With this in mind, the ongoing study will concentrate on the phosphorus removal efficiency of *Canna lily* and *Cyperus alternifolius*, both of which have shown encouraging results in terms of nutrient removal. Another key goal of this research is to learn more about the various fractions of phosphate in wastewater and plants and their translocation in various parts of the plant species.

In this present study both *Canna lily* and *Cyperus alternifolius* planted in two separate pilot-scale vertical flow constructed wetlands (VFW) in the campus of Delhi Technological

University. Both CW cells (*Canna lily* and *Cyperus alternifolius*) had a 38 cm thick layer of sand-gravel substrate. Both cells were the same size, with a cylindrical tank constructed of high-density polyethylene (HDPE) plastic and a substrate depth of 43 cm and a diameter of 55 cm. Water was kept at a height of 5 cm above the gravel surface. The effluent was collected from the tap provided at the bottom of the tank, and the influent was fed from the top. For plant stabilization, CW cells were amended with the fertilizers di-ammonium phosphate (DAP) and urea. The investigation began once the plants had been adopted and stabilized in their new habitat. The experiment ran from December 2021 to May 2022, with the aim of determining the impact of seasonal change and nutrient influent concentrations on plant growth, health, and nutrient removal efficiency. It should be emphasized that the influent should be high in nitrogenous materials; otherwise, the shortfall will exacerbate with the low temperature, putting the plant under stress. *Canna lily* and *Cyperus alternifolius* removal behaviour was heavily influenced by weather variables, particularly temperature, rainfall and humidity. The rate of evapotranspiration was influenced by temperature; the higher the temperature, the higher the rate of evapotranspiration, which results in an increased/uptake of water along the nutrients. Sunlight hours also influence the photosynthesis process and plant output. Summer time brought more sunshine, which enhanced productivity and nutrient uptake from wastewater. As a result, in the present study, removal efficiency for total phosphate in *Canna lily* followed the trend of increasing order from winter season (77.6 %), spring season (82.6 %), and summer season (87.79 %). In the similar order winter season (62.35 %) < spring season (74.24 %) < summer season (81.49 %), for *Cyperus alternifolius* CW cell showed the similar trend. During the current study, the intake phosphate concentration was kept constant at PO_4^3P = 5 mg/l. *C. lily* TP effluent concentrations throughout the study ranged from 0.18 mg/l to 1.82 mg/l. The average TP effluent concentrations in *C. alternifolius* ranged from 0.33 mg/l to 2.65 mg/l. The accumulation of TP in various parts of plant tissue were investigated in order to determine the overall effect on the plants (C. lily and C. alternifolius) over the study period. The concentration of phosphate (mg/g) in roots, stems, leaves, and flowers has been analyzed, with the highest accumulation of phosphate in leaves, followed by flowers, stems, and roots, indicating that phosphate will play a role in the plant's metabolism and growth. The increased root length, soot length, and plant density further indicate that the waste water application had no negative impact on both plants. The current study concludes that both *C. lily* and *C. alternifolius* are advanced and promising tools for phosphate removal from wastewater, but *C. lily* has performed better than *C. alternifolius* in all seasons.

Though both *C. lily* and *C. alternifolius* have shown good potential for removing phosphate from wastewater in a pilot-scale study over a short period of time (Dec'21-May'22). The efficiency of removal may vary in the field due to the varied nature of chemical species, their interactions, temperature variation, and humidity variation throughout the year. Heavy metals and pesticides in wastewater might stunt plant growth and lead to decreased removal rates. As a result, the research might be expanded to look at the effect as well as the removal efficiency in real-world conditions with large temperature and humidity fluctuations.

REFERNCES

Bailey, L. H, (1962). The Standard Enycyclopedia of Horticulture Vol2. Mac Mill Lct. Co.

Barley RW, Hutton C, Brown, MME, Cusworth JE, Hamilton TJ. Trends in biomass and metal sequestration associated with reeds and algae at Wheel Jane Biorem pilot passive treatment plant. Sci Tot Environ. 2005; 338 (1–2): 107–14.

Belmont, Marco & Metcalfe, Chris. (2003). Feasibility of using ornamental plants (Zantedeschia aethiopica) in subsurface flow treatment wetlands to remove nitrogen, chemical oxygen demand and nonylphenol ethoxylate surfactants - A laboratory-scale study. Ecological Engineering. 21. 233-247.

Brix H. Wastewater treatment in constructed wetlands: systems design, removal processes, and treatment performance. In: Moshiri GA, editor. Constructed wetlands for water quality improvement. Boca Raton, Florida: CRC press; 1993. p.9-22.

C. Le, Y. Zha, Y. Li, D. Sun, H. Lu, B. Yin, “Eutrophication of Lake Waters in China: Cost, Causes, and Control”, Environmental Management, Vol. 45, No. 4, pp. 662-668, 2010.

Choudhary et al. (2011) performance of constructed wetland for the treatment of pulp and paper mill wastewater. Proceedings of World Environmental and Water resources congress 2011: Bearing Knowledge for sustainability, Palm springs, California, USA, p-4856-4865,22-26 May.

Correll, D. L. "Phosphorus: a rate limiting nutrient in surface waters." Poultry science 78.5 (1999): 674-682.

DeBusk WF. Wastewater Treatment Wetlands: Applications and Treatment Efficiency. A fact sheet of the Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. 1999.

"Department of Environmental Protection State of Florida Glossary". State of Florida. Retrieved 2011-09-25.

Dorgham, M. M. (2014). Effects of eutrophication. In Eutrophication: Causes, consequences and control (pp. 29-44). Springer, Dordrecht.

EPA. Design Manual: Constructed Wetlands Treatment of Municipal Wastewaters. EPA/625/R-99/010, 2000.

Frank, I.B., P. Lundgren and P. Falkowski, 2003. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Research in Microbial., 154: 157-164.

Gardner, T., Geary, P., Gordon, I., 1997, Ecological sustainability and on-site effluent treatment systems. Aust.J. Environ.Manage.

Gersberg, Richard & Elkins, B.V. & Lyon, Stephen & Goldman, Charles. (1986). Role of Aquatic Plants in Wastewater Treatment by Artificial Wetlands. Water Research. 20. 363-368.

Gersberg, RM, Greatheart RA, Ives M. Pathogens removal in constructed wetlands: In Hammer DA, editor. Constructed wetlands for wastewater treatment. Chelsea, Michigan: Lewis Publishers; 1989. p. 431-45.

Gopal, B. Natural and Constructed Wetlands for Wastewater Treatment: Potentials and Problems. Water Sci. Technol. 1994, 40, 27–35.

Grace, J. B. and J. S. Harrison. 1986. The biology of Canadian weeds. 73. Typha latifolia L., Typha angustifolia L. and Typha xglauca Godr. Can. J. Plant Sci. 66:361-379.

Gunn, J. M., Snucins, E. D., Yan, N. D., & Arts, M. T. (2001). Use of water clarity to monitor the effects of climate change and other stressors on oligotrophic lakes. Environmental Monitoring and Assessment, 67(1), 69-88.

Haritash, A.K., Dutta, S. & Sharma, A. Phosphate uptake and translocation in a tropical Canna-based constructed wetland. Ecol Process 6, 12 (2017).

Hasegawa, A; M. Hashimoto; S. Fukai, and A. Tajima, (1998 a). Dwarf Cyperus papyrus, L. for potted plants. Acta Hort., 454: 191-194.

Herath, I.; Vithanage, M. Phytoremediation in Constructed Wetlands. In Phytoremediation; Springer International Publishing: Cham, Switzerland, 2015; pp. 243–263.

Higgins JP, Hurd S., Weil C. The use of engineered wetlands to treat recalcitrant wastewaters. J. Enviro. Sci. Health, A 2000; 35(8), 1309-1334.

Hua, S.C. The Use of Constructed Wetlands for Wastewater Treatment; Wetlands International—Asia Pacific: Selangor, Malaysia, 2003.

Jan Vymazal, 2005. Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Volume 25, Issue 5.

Jan Vymazal, 2009. The use of constructed wetlands with horizontal sub-surface flow for various types of wastewaters. Ecological Engineering, Volume 35, Issue 1.

Jones, M. B. and S. W. Humphries, (2002). Impacts of the C4 sedge Cyperus papyrus. L on carbon and water fluxes in an African wetland Hydrobiology. November 2002, volume 488, Issue, 1-3.

Kadlec RH, Knight RL. Treatment Wetlands. New York: Lewis Publishers, CRC: 1996.

Keddy, Paul A. (2010). Wetland ecology: principles and conservation (2nd ed.). New York: Cambridge University Press. p. 497. ISBN 978-0521519403.

Khan.M (2014). Eutrophication: Challenges and Solutions. 10.13140/2.1.3673.8884.

McCutcheon S C, Jorgensen S E. Phytoremediation. Ecological engineering 2008; 2751-2766.

Mitch, L. M. 2000. Common cattail, Typha latifolia L. Weed Technology 14:446-450.

McCutcheon S C, Jorgensen S E. Phytoremediation. Ecological engineering 2008; 2751-2766.

Mohammad Badruzzaman, Jimena Pinzon, Joan Oppenheimer, Joseph. G. Jacangelo, Sources of nutrients impacting surface waters in Florida: A review, Journal of Environmental Management, Volume 109, 2012, Pages 80-92.

Motivans, K. and S. Apfelbaum. 1987. Element stewardship abstract for Typha spp., North American cattails. The Nature Conservancy. (October 2007).

Neralla S, Weaver RW, Lesikar BJ, Persyn RA. Improvement of domestic wastewater quality by subsurface flowconstructed wetlands, Bio Technol. 2000; 75: 19-25.

Noller BN, Woods PH, Ross BJ. Case studies of wetland filtration of mine waste water in constructed and naturally occurring systems in northern Australia. Wat Sci and Technol. 1994; 29: 257-66.

Ottova V, Balcarova J, Vymazal J. Microbial characteristics of constructed wetlands. Wat Sci Technol. 1997; 35: 117-23.

P. Rajasulochana, V. Preethy, Comparison on efficiency of various techniques in treatment of waste and sewage water – A comprehensive review, Resource-Efficient Technologies, Volume 2, Issue 4, 2016, Pages 175-184.

Pandey, J.; Chauhan, A.; Jain, R.K. Integrative Approaches for Assessing the Ecological Sustainability of in Situ Bioremediation. FEMS Microbiol. Rev. 2009, 33, 324–375.

Piyachomkwan K., Chotineeranat S., Kijkhunasatian. C., Tonwitowat R., Prammanee S., Oates, C.G., & Sriroth K., 2002. Edible canna (Canna edulis) as a complementary starch source to cassava for the starch industry. Ind. Crop. Prod. 16:11-21.

Prasad, S.N., Ramachandra, T.V., Ahalya, N., Sengupta, T., Kumar, A., Tiwari, A.K., Vijayan, V.S., Vijayan, L., 2002. Conservation of wetlands of India – a review. Trop. Ecol. 43 (1), 173–186.

Richardson, JL, Arndt, JL & Montgomery, JA 2001, ‘Hydrology of wetland and related soils’ in JL Richardson & MJ Vepraskas (eds), Wetland Soils, Lewis Publishers, Boca Raton.

R.S.S Wu, Eutrophication, Water Borne Pathogens and Xenobiotic Compounds: Environmental Risks and Challenges, Marine Pollution Bulletin, Volume 39, Issues 1–12, 1999, Pages 11-22.

Sasse L., 1998. Decentralized Wastewater Treatment in Developing Countries. Bre-men, Germany.

Sheoran AS, Sheoran V. Heavy metal removal mechanism of acid mine drainage in wetlands: A critical review. Min Engg. 2006; 19: 105–16.

Sinicrope TL, Langis R, Gersberg RM, Busanardo MJ, Zedler JB. Metal removal by wetland mesocosms subjected to different hydroperiods. Ecol Engg. 1992; 1(4): 309-22.

Sobolewski A. A review of processes responsible for metal removal in wetlands treating contaminated mine drainage. Int J Phytor. 1999; 1(1): 19–51.

Sornvoraweat, B., Kongkiattikajorn, J., 2011. Separated hydrolysis and fermentation of water hyacinth leaves for ethanol production. J. Life Sci. 5, 212–219.

Stevens, M. and C. Hoag. 2000. NRCS plant guide: Narrowleaf cattail, Typha angustifolia L. USDA, NRCS, National Plant Data Center and Idaho Plant Materials Center. (October 2007).

Stottmeister U, Kuschk P, Kappelmeyer U, Kastner M, Bederski O et al. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotech Adv. 2003; 22: 93– 17.

US EPA. (2015). A Compilation of Cost Data Associated with the Impacts and Control of Nutrient Pollution. US EPA

Vymazal, J & Kropfleova, L 2008, ‘Wastewater treatment in constructed wetlands with horizontal sub-surface flow’, Environmental Pollution, Vol. 14.

Walker DJ, Hurl S. The reduction of heavy metals in a storm water wetland. Ecol Engg. 2002; 18 (4): 407-14.

Wang X, Wang Y, Tian J, Lim BL, Yan X, Liao H (2009) Overexpressing AtPAP15 enhances phosphorus efficiency in soybean. Plant Physiol 151:233–240.

Wolverton, B.C., 1990. Aquatic plant/microbial filters for treating septic tank effluent. In: Hammer, D.A. (Ed.), Constructed Wetlands for Wastewater Treatment. Lewis Publishers, NY.

Worsfold, P. J.; Monbet, P.; Tappin, A. D.; Fitzsimons, M. F.; Stiles, D. A.; McKelvie, I. D. Characterisation and quantification of organic phosphorus and organic nitrogen components in aquatic systems: a review. Anal. Chim. Acta 2008, 624 (1), 37−58.

Annexure I

Ambient temperature profile during the study

Date	Maximum Temperature (°C)	Minimum Temperature (°C)	Average Temperature (°C)	Rainfall (mm)	*Canna lily*				*Cyperus alternifolius*			
					AP (mg/l)		TP (mg/l)		AP (mg/l)		TP (mg/l)	
					Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
01-12-2021	22.0	14.0	18.0	0.0	4.8	1.1	5.2	1.7	4.8	1.7	5.2	2.7
02-12-2021	19.0	15.0	17.0	6.5	4.9	0.7	5.2	1.2	4.9	1.5	5.2	2.2
03-12-2021	22.0	14.0	18.0	0.0	5.7	0.7	5.9	1.3	5.7	1.2	5.9	1.7
06-12-2021	24.0	15.0	19.5	0.0	5.4	0.9	6.0	1.3	5.4	1.5	6.0	1.7
08-12-2021	23.0	13.0	18.0	0.0	4.1	0.5	4.6	0.9	4.1	1.9	4.6	2.3
09-12-2021	23.0	11.0	17.0	0.0	5.0	0.7	5.0	1.2	5.0	1.9	5.0	2.3
10-12-2021	22.0	12.0	17.0	0.0	5.1	0.9	5.7	1.1	5.1	1.7	5.7	2.1
13-12-2021	22.0	9.0	15.5	0.0	4.6	0.7	4.0	1.2	4.6	1.7	4.0	2.1
15-12-2021	21.0	11.0	16.0	0.0	5.2	1.0	5.6	1.4	5.2	1.1	5.6	1.5
16-12-2021	21.0	12.0	16.5	0.0	4.8	0.8	5.0	1.6	4.8	1.7	5.0	2.0
17-12-2021	18.0	8.0	13.0	0.0	5.0	0.9	5.1	1.7	5.0	1.5	5.1	2.2
20-12-2021	20.0	6.0	13.0	0.0	5.0	0.8	5.2	1.1	5.0	1.8	5.2	2.1
22-12-2021	22.0	8.0	15.0	0.0	4.8	0.8	5.4	1.1	4.8	1.8	5.4	2.2
23-12-2021	22.0	10.0	16.0	0.0	5.1	0.7	5.3	0.9	5.1	2.3	5.3	2.7
24-12-2021	22.0	10.0	16.0	0.0	4.5	0.9	4.7	1.2	4.5	2.3	4.7	2.5
27-12-2021	22.0	12.0	17.0	3.1	4.3	0.9	4.4	1.1	4.3	1.8	4.4	2.2
29-12-2021	20.0	10.0	15.0	0.0	5.4	0.9	5.8	1.1	5.4	2.0	5.8	2.3
30-12-2021	19.0	7.0	13.0	0.0	5.2	0.9	5.8	1.3	5.2	1.8	5.8	2.3
31-12-2021	18.0	7.0	12.5	0.0	5.2	0.6	5.7	0.6	5.2	2.0	5.7	2.4
03-02-2022	15.0	11.0	13.0	0.0	4.8	0.6	5.2	0.7	4.8	0.3	5.2	0.3
09-02-2022	22.0	12.0	17.0	0.0	4.9	0.6	5.3	0.6	4.9	1.1	5.3	1.5

10-02-2022	**22.0**	**10.0**	**16.0**	**0.0**	**5.2**	**0.7**	**5.6**	**0.7**	**5.2**	**1.2**	**5.6**	**1.9**
11-02-2022	**22.0**	**9.0**	**15.5**	**0.0**	**5.9**	**0.8**	**6.5**	**1.1**	**5.9**	**0.9**	**6.5**	**1.0**
14-02-2022	**26.0**	**12.0**	**19.0**	**0.0**	**5.4**	**0.8**	**5.7**	**1.0**	**5.4**	**1.6**	**5.7**	**1.9**
15-02-2022	**26.0**	**12.0**	**19.0**	**0.0**	**5.3**	**0.8**	**5.6**	**1.0**	**5.3**	**1.0**	**5.6**	**1.2**
16-02-2022	**26.0**	**13.0**	**19.5**	**0.0**	**4.5**	**0.6**	**4.7**	**0.8**	**4.5**	**0.7**	**4.7**	**1.3**
17-02-2022	**27.0**	**14.0**	**20.5**	**0.0**	**4.6**	**0.6**	**4.0**	**0.7**	**4.6**	**1.3**	**4.0**	**1.4**
18-02-2022	**28.0**	**13.0**	**20.5**	**0.0**	**4.8**	**0.8**	**5.3**	**1.3**	**4.8**	**1.5**	**5.3**	**1.9**
22-02-2022	**26.0**	**15.0**	**20.5**	**0.0**	**4.8**	**0.8**	**5.5**	**1.5**	**4.8**	**1.5**	**5.5**	**1.8**
23-02-2022	**24.0**	**16.0**	**20.0**	**0.0**	**4.4**	**0.7**	**5.1**	**1.2**	**4.4**	**1.8**	**5.1**	**2.0**
24-02-2022	**27.0**	**15.0**	**21.0**	**0.0**	**4.8**	**0.8**	**5.5**	**1.4**	**4.8**	**0.9**	**5.5**	**1.5**
25-02-2022	**27.0**	**16.0**	**21.5**	**0.0**	**4.6**	**0.8**	**4.9**	**1.0**	**4.6**	**1.1**	**4.9**	**1.5**
04-03-2022	**26.0**	**16.0**	**21.0**	**0.0**	**3.3**	**0.7**	**3.7**	**0.9**	**3.3**	**0.9**	**3.7**	**1.2**
05-03-2022	**26.0**	**14.0**	**20.0**	**0.0**	**5.8**	**0.6**	**6.2**	**0.9**	**5.8**	**0.8**	**6.2**	**1.3**
07-03-2022	**30.0**	**16.0**	**23.0**	**0.0**	**4.3**	**0.7**	**5.0**	**1.1**	**4.3**	**1.2**	**5.0**	**1.7**
08-03-2022	**31.0**	**17.0**	**24.0**	**0.0**	**4.7**	**0.7**	**5.3**	**0.7**	**4.7**	**1.0**	**5.3**	**1.0**
09-03-2022	**31.0**	**17.0**	**24.0**	**0.0**	**5.6**	**0.2**	**6.0**	**0.5**	**5.6**	**0.8**	**6.0**	**1.2**
10-03-2022	**30.0**	**16.0**	**23.0**	**0.0**	**5.6**	**0.2**	**6.2**	**0.8**	**5.6**	**0.7**	**6.2**	**1.2**
11-03-2022	**29.0**	**16.0**	**22.5**	**0.0**	**4.3**	**0.7**	**4.9**	**0.7**	**4.3**	**0.8**	**4.9**	**1.0**
14-03-2022	**34.0**	**19.0**	**26.5**	**0.0**	**5.0**	**0.6**	**5.7**	**0.8**	**5.0**	**0.9**	**5.7**	**1.1**
15-03-2022	**35.0**	**20.0**	**27.5**	**0.0**	**5.0**	**0.4**	**5.5**	**0.7**	**5.0**	**1.0**	**5.5**	**1.1**
16-03-2022	**35.0**	**21.0**	**28.0**	**0.0**	**5.2**	**0.7**	**6.1**	**0.8**	**5.2**	**1.1**	**6.1**	**1.3**
21-03-2022	**36.0**	**23.0**	**29.5**	**0.0**	**4.2**	**0.7**	**4.5**	**0.8**	**4.2**	**0.9**	**4.5**	**1.1**
23-03-2022	**35.0**	**22.0**	**28.5**	**0.0**	**5.0**	**0.8**	**5.9**	**1.0**	**5.0**	**1.0**	**5.9**	**1.3**
24-03-2022	**35.0**	**22.0**	**28.5**	**0.0**	**4.5**	**0.6**	**5.3**	**0.7**	**4.5**	**1.2**	**5.3**	**1.3**
25-03-2022	**35.0**	**21.0**	**28.0**	**0.0**	**4.5**	**0.6**	**5.0**	**0.6**	**4.5**	**1.0**	**5.0**	**1.0**
28-03-2022	**39.0**	**21.0**	**30.0**	**0.0**	**5.2**	**0.7**	**5.4**	**1.0**	**5.2**	**1.3**	**5.4**	**1.5**
29-03-2022	**39.0**	**21.0**	**30.0**	**0.0**	**4.2**	**0.6**	**5.0**	**0.9**	**4.2**	**1.1**	**5.0**	**1.4**
31-03-2022	**40.0**	**24.0**	**32.0**	**0.0**	**5.1**	**0.7**	**5.5**	**0.9**	**5.1**	**1.0**	**5.5**	**1.2**
01-04-2022	**37.0**	**22.0**	**29.5**	**0.0**	**4.7**	**0.4**	**5.2**	**0.6**	**4.7**	**0.7**	**5.2**	**1.1**

06-04-2022	**41.0**	**22.0**	**31.5**	**0.0**	**5.6**	**0.4**	**6.0**	**0.6**	**5.6**	**1.2**	**6.0**	**1.1**
07-04-2022	**40.0**	**23.0**	**31.5**	**0.0**	**5.2**	**0.6**	**5.9**	**0.7**	**5.2**	**1.1**	**5.9**	**1.3**
08-04-2022	**42.0**	**24.0**	**33.0**	**0.0**	**6.0**	**0.4**	**6.1**	**0.4**	**6.0**	**0.4**	**6.1**	**0.5**
11-04-2022	**43.0**	**26.0**	**34.5**	**0.0**	**5.6**	**0.7**	**6.2**	**0.8**	**5.6**	**0.8**	**6.2**	**1.0**
12-04-2022	**39.0**	**27.0**	**33.0**	**0.0**	**5.4**	**0.5**	**5.9**	**0.6**	**5.4**	**0.9**	**5.9**	**1.1**
14-04-2022	**40.0**	**27.0**	**33.5**	**0.0**	**5.3**	**0.2**	**6.0**	**0.3**	**5.3**	**0.3**	**6.0**	**0.3**
18-04-2022	**42.0**	**26.0**	**34.0**	**0.0**	**4.9**	**0.9**	**5.4**	**0.9**	**4.9**	**1.3**	**5.4**	**1.5**
19-04-2022	**42.0**	**26.0**	**31.5**	**0.0**	**4.8**	**0.9**	**5.4**	**1.0**	**4.8**	**1.1**	**5.4**	**1.5**
20-04-2022	**43.0**	**26.0**	**33.5**	**0.0**	**5.0**	**0.8**	**5.4**	**0.9**	**5.0**	**1.3**	**5.4**	**1.5**
21-04-2022	**41.0**	**27.0**	**31.5**	**0.0**	**5.0**	**0.7**	**5.5**	**0.5**	**5.0**	**1.1**	**5.5**	**1.4**
22-04-2022	**36.0**	**27.0**	**32.0**	**0.0**	**5.0**	**0.3**	**5.7**	**0.5**	**5.0**	**0.7**	**5.7**	**0.7**
25-04-2022	**40.0**	**24.0**	**32.5**	**0.0**	**6.1**	**1.6**	**6.8**	**1.8**	**6.1**	**1.4**	**6.8**	**1.5**
26-04-2022	**41.0**	**25.0**	**36.0**	**0.0**	**5.6**	**0.4**	**6.2**	**0.5**	**5.6**	**0.6**	**6.2**	**0.9**
27-04-2022	**41.0**	**26.0**	**36.0**	**0.0**	**6.2**	**0.4**	**6.4**	**0.5**	**6.2**	**0.5**	**6.4**	**0.6**
29-04-2022	**44.0**	**28.0**	**36.0**	**0.0**	**5.9**	**0.2**	**6.0**	**0.3**	**5.9**	**0.2**	**6.0**	**0.6**
02-05-2022	**40.0**	**27.0**	**33.5**	**0.0**	**6.3**	**0.2**	**7.0**	**0.2**	**6.3**	**0.5**	**7.0**	**0.6**
04-05-2022	**40.0**	**25.0**	**32.5**	**0.0**	**4.9**	**0.3**	**5.5**	**0.3**	**4.9**	**0.4**	**5.5**	**0.6**
05-05-2022	**39.0**	**25.0**	**32.0**	**14.8**	**5.9**	**0.2**	**6.3**	**0.1**	**5.9**	**0.6**	**6.3**	**0.7**
06-05-2022	**38.0**	**27.0**	**32.5**	**0.0**	**6.3**	**0.3**	**6.8**	**0.3**	**6.3**	**0.5**	**6.8**	**0.5**
09-05-2022	**41.0**	**29.0**	**35.0**	**0.0**	**5.5**	**0.2**	**6.3**	**0.3**	**5.5**	**0.6**	**6.3**	**0.8**
10-05-2022	**42.0**	**29.0**	**35.5**	**0.0**	**5.3**	**0.3**	**5.9**	**0.4**	**5.3**	**0.6**	**5.9**	**0.6**
Min.	**15.0**	**6.0**	**12.5**	**0.0**	**3.3**	**0.2**	**3.7**	**0.1**	**3.3**	**0.2**	**3.7**	**0.3**
Max.	**44.0**	**29.0**	**36.0**	**14.8**	**6.3**	**1.6**	**7.0**	**1.8**	**6.3**	**2.3**	**7.0**	**2.7**
Average ± SD	**30.7 ± 8.5**	**17.8 ± 6.7**	**24.3 ± 7.5**	**0.3 ± 1.9**	**5.1 ± 0.6**	**0.6 ± 0.3**	**5.5 ± 0.6**	**0.9 ± 0.4**	**5.1 ± 0.6**	**1.1 ± 0.5**	**5.5 ± 0.6**	**1.4 ± 0.6**