

# **POTENTIAL OF DIFFERENT AQUATIC MACROPHYTES TOWARDS PHOSPHATE REMOVAL IN A CONSTRUCTED WETLAND**

A MAJOR PROJECT DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE  
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## **MASTER OF TECHNOLOGY IN ENVIRONMENTAL ENGINEERING**

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**JUNE , 2019**

## **CANDIDATE'S DECLARATION**

I Priyanka singh, 2K17/ENE/11 student of M.Tech, Environmental Engineering, hereby declare that the project dissertation entitled **“POTENTIAL OF DIFFERENT AQUATIC MACROPHYTES TOWARDS PHOSPHATE REMOVAL IN A CONSTRUCTED WETLAND”** which is submitted by me to the Department of Environmental Engineering, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of degree of Master of technology, is original not copied from any source without paper citation. The work has not been submitted in part or full for the award of degree for diploma in this or any other institute.

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**CERTIFICATE**

This is to certify that the project report entitled “**POTENTIAL OF DIFFERENT AQUATIC MACROPHYTES TOWARDS PHOSPHATE REMOVAL IN A CONSTRUCTED WETLAND**” is an authentic report of the major project done in the partial fulfilment of the requirement for the award of the degree of Master of Technology in Environmental Engineering from Delhi Technological University during the year 2019.

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## Abstract

Growth of phytoplankton in a water body is limited by the concentration of nutrients (Phosphate and Nitrogen) in that water body. Excessive growth of algae will lead to serious issues like eutrophication. Constructed Wetlands (CW) are an effective technology which is widely used to remove nutrients from waste water. The present study had been undertaken in three wetlands that were constructed in the campus of Delhi Technological University with three different types of vegetation in it, to obtain the potential of plants to remove phosphate from waste water. Study was started in the month of June of 2018 and continued till March of 2019. The variation in the percentage removal with ambient temperature was monitored. Percentage removal of available phosphate (AP), and total phosphate (TP) in *C. indica*, and *P. australis* based cells was varied from 78 to 25%, and 71 to 31%, and 96 to 33% and 58 to 25%, respectively. AP removal in *I. indica* based cell was varied from 54 to 20%. Percentage removal of ammonia was also been monitored in all three wetland cells and it was varied from 91 to 65%, 94 to 81%, and 97 to 70%, for *C. indica*, *I. indica* and *P. australis*, respectively. Bioaccumulation in plants (for all three plants), trend of nutrient translocation in different parts of plant (for *C. indica*) with season, and role of substrate in wetlands were also performed in this study. It was observed that stems allocate higher amount of phosphate than leaves and flowers in *C. indica*, but relative accumulation of phosphate was observed higher in flowers. Accumulation of phosphate by *I. indica* was found minimum due to low growth of the plant at available temperature. From the harvested tissue of *C. indica* it was observed that during growth period nutrient translocation is generally from bottom to top i.e. from roots to flowers but in fall (autumn and winter) reverse translocation occurs i.e. from flowers to roots. In the sediments of CW1 (*C. indica*) presence of non-apatite phosphate was found higher than apatite phosphate, which indicates stable form of phosphate. All three plants were found efficient to remove nutrients from waste water, although *I. indica* was found less adaptive to natural shocks than *C. indica* and *P. australis*.

# CHAPTER -1

## INTRODUCTION

**CHAPTER -2**

**REVIEW OF LITERATURE**

## CHAPTER -3

# MATERIALS AND METHODS

# CHAPTER -4

## RESULTS AND DISCUSSION

**CHAPTER -5**  
**CONCLUSION**

## INTRODUCTION

Agricultural runoff containing nitrogen and phosphorous is a major source of eutrophication in a surface water body. Nitrogen and phosphorous are found to be limiting elements for algal growth in most of freshwater ecosystems, so it become necessary to remove them before they get enter into the freshwater body. The drawbacks of conventional treatments are high cost associated, high sludge formation and low removal efficiency for nutrients. Techniques like chemical precipitation, ion exchange, and reverse osmosis required huge energy and are costly (Jiang et al. 2008; Haritash et al. 2015). Among the low cost nonconventional technology for nutrient removal from wastewater, constructed wetlands (CW) have been found to be effective in removal of nutrients from agricultural wastewater (Gupta et al. 2016; Li et al. 2014, Wu et al. 2011). CWs are widely being used to treat agricultural runoff from small towns and villages for the protection of surface water bodies from eutrophication (Wu et al. 2009). CW technology purposes like water storage is very useful particularly in rural and mountainous areas where construction of a centralized conventional is not feasible (Wu et al. 2018). Constructed wetlands are systems that have been designed to utilize the natural processes in treating wastewaters. They are designed to take advantage of many of the same processes that occur in natural wetlands, but do so within a more controlled environment (Vymazal 2010). They are widely used for various, groundwater replenishment, shoreline stabilisation, water purification, reservoirs of biodiversity, wetland products, climate change mitigation etc. Using non-food crops as part of a wastewater treatment system can also have the added benefit of generating materials for the production of biofuels (Yang et al. 2008). Constructed wetlands can be classified on the basis of water hydrology and vegetation, type of flow, and direction of flow. The different types of constructed wetlands for wastewater treatment can be categorized as free water surface (FWS) wetlands and subsurface flow (SSF) wetlands on the basis of water hydrology and they are further classified as emergent, submerge plants or floating leaved wetlands depending on the vegetation type. SSF wetlands are preferred over FWS wetland because they have higher treatment efficiency per unit area of land, microbial processes occur in the root zone even in cold climates

however higher capital cost is also associated with SSF wetlands (Greenway 2003). Subsurface wetlands are subdivided into horizontal flow (HF) and vertical flow (VF) on the basis of direction of flow and availability of oxygen. The amount of oxygen is greater in a VF beds than in HF beds. As VF wetlands have higher oxygen availability, they are preferred for P removal over HF wetlands (Vyzamal 2007).

Characteristics of a eutrophic water body changes with season, during summer when wind speed is minimum and oxygen demand is high surface sediment may become anaerobic, this will cause release of phosphate in the water column (Moore and Reddy 1994). This required a treatment technology that has high efficiency during summer. Performance of a wetland system is depended on environmental parameters, such as temperature, dissolve oxygen, redox potential, pH etc. (Wu et al. 2018). All the processes for removal of nutrients vary with change in physico-bio-chemical parameters in water. Oxygen is required to transforms ammonical nitrogen ( $\text{NH}_4^+\text{-N}$ ) into nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), plant and other microphytes take nitrate as nitrogen source for their growth. pH of the pore water also governs the transformation of nitrogen in a wetland system, nitrogen emitted as nitrous oxide which is a greenhouse gas at low pH (<6) whereas at higher pH atmospheric N is form (Verhoeven and Meuleman 1999) and if pH of pore water is above 8.0 ammonia will form (Vymazal 2007). Substrate in a constructed wetland plays multiple role, they act as filter media for the suspended solids; adsorbent site for phosphate; mechanical support for emergent plant etc. Effective size of the media affect the removal of nutrient as gravel base wetlands are more efficient than soil based wetlands for transformation of ammonia due to their high porosity and high microbial growth (Yang et al. 2001, Haritash et al. 2017). Sediments chemistry too influence the removal of nutrients, especially phosphate, calcium and magnesium present in sediments make strong bond with phosphate under alkaline conditions, whereas iron and aluminium can dominantly remove phosphate at neutral pH (Haritash et al. 2017). Common processes of phosphate removal are sedimentation, adsorption, filtration, microbial degradation, plant uptake etc., whereas for nitrogen removal nitrification, denitrification, ammonification, microbial degradation, and plant uptake are the related processes. Physicochemical processes, such as the fixation of phosphate by calcium and iron in the substrate are the major source and sink of phosphate in a CW (Haritash et al. 2015) but fixation of phosphate through adsorption on soil surface



has a saturation value above which no nutrient will further adsorb on the surface of the media (Avishek et al. 2011). Furthermore, little is known about change in nutrient uptake with different weather conditions. Plants in a CW play very crucial role as they are responsible for plethora of biological, chemical and physical processes, and are significantly contributed to wastewater purification. Plants are the major component of a wetland system as more than 50% removal of pollutant is done by plant through various biochemical processes. Different plant has different removal efficiency within same or different physico-chemical conditions, and it is important to identify most suitable plant in the available condition. Study based on removal of nutrients using same plant in different physico-chemical conditions and different plant in same physico-chemical conditions has been focused more in recent years. Aquatic macrophytes can utilize large amounts of nitrogen and phosphorus and thus remove them from the water. They are considered as main key component of a wetland system as they take up nutrients from waste water for their growth and enhance purification through various chemical and microbial processes (Wu et al. 2011). The nutrient removal from the waste water depends upon the plant type, growth rate, nutrition composition, water uptake of the plant etc. (Zhang 2007). Emergent plant uptake nutrients from soil substrate whereas floating plant uptake nutrients from water column. Emergent plants which have water root such as *Phragmites australis* can uptake nutrients from water also (Nilusha 2017). The selection of plant species depends upon the local availability of species; the physical structure of the constructed wetland (floating, emergent or submerged type); and the chemical composition of the wastewater effluent. Since inorganic nitrogen and phosphorus are essential for plant growth, it is possible to maximise the amount of nutrients removed from wastewater effluent by selecting macrophytes with a high capacity for nutrient absorption and conversion to organic plant biomass. The efficiency of nutrient uptake by plants from soil is affected by several plant factors such as; root geometry radius, length, density and numbers of root hairs, the transport kinetic parameters, root exudates and the adaptability of the roots to the soil microbes (Nielsen 1979). In the present study plants selected to treat waste water were; *Phragmites australis* (common reed), *Canna indica*, and *Ipomoea indica*. *P. australis* is an emergent plant widely used in constructed wetlands for treatment of wastewater, due to its high growth rate and great capacity for nutrient accumulation in its stems, roots,

and rhizomes (Vyamazal 2005). Other advantages of reed are ability to adapt its surrounding environment and tolerance toward levels of nutrient supply (Ruiz and Valasco 2009; Wathugala et al. 1987). It is capable of releasing antibiotics phyto-metallophores and phytochelatins which reduces the microbial activity in the substrate (Vincent et al. 1994). *C. indica* is a perennial plant and allocates the majority of its biomass to shoots; it is possible to regularly harvest and remove biomass from the treatment system (Chen et al. 2009, Haritash et al. 2017). Due to its higher growth rate and high biomass production *C. indica* has found to be having high nutrient uptake, and tolerance to water stress which makes it suitable for phytoremediation (Haritash et al. 2015). *C. indica* has a higher water demand in order of 1.4 liters/plant/day (Chen et al. 2009). *I. indica* (blue morning glory) is a fast growing herbaceous vine, smothered other species due to its creeping property (Greenway 2003). The stems and leaves of emergent macrophytes and the roots of floating plants reduce water velocity and turbulence causing filtration and settlement of particles; and provide an increased surface area for the attachment of epiphytic algae and microorganisms (Greenway 2003). This study has been done to observe the variations in the percentage removal efficiency of different wetland plants and to monitor factor affecting it. Plants selected for this study was *Canna indica*, *Ipomoea indica*, and *Phragmites australis* with sand and gravel packed substrate in *C. indica* and gravel packed substrate for *I. indica* and *P. australis*.

### **Objectives of this study**

1. To monitor the environmental factors controlling nutrient removal.
2. Analyse the seasonal variation in the removal of nutrients in synthetic waste water from three vertical flow constructed wetlands containing non-mixed culture of *Phragmites australis*, *Ipomoea indica*, and *Canna indica* plants, individually.
3. To compare the bioaccumulation of Phosphate by the plants, and to observe the trend of Phosphate accumulated in dead tissue of *C. indica* plant in different growth cycles.
4. To find the role of sediment chemistry, in the wetland system, towards phosphate removal.

## REVIEW OF LITRATURE

Waste water treatment by wetlands was first experimented in 1950s but a fully constructed wetland with free water surface was built in 1967 in Netherlands. Constructed wetlands have been proven as an economic technology for waste water treatment but require more area as compared to conventional treatments. Modification in this technology to reduce its area demand is the concern of researchers. Bioavailability of phosphate is depended on the biochemistry of sediments, which is affected by factor like redox (Eh) potential and pH. The effects of Eh and pH was studied on sediments (characterised as mud) of Lake Okeechobee. Sediments and water slurries were mixed in fixed proportion to get required Eh (500, 250, 0, and -25 mV) and pH (5.5, 6.5, 7.5, and 8.5) levels. It was observed that at positive Eh no phosphate was released from sediments in sample having slightly basic pH (7.5 and 8.5) and at higher redox potential effect of pH is negligible (Moore and Reddy 1994). Plants are important components of a CW system, they directly uptake pollutants from water but at a certain level. High salt in water can cause stress conditions on the growth of plants (Romero et al. 1999). Effects of ammonia and phosphate on the growth, biomass, nutrient allocation and ammonium kinetics were studied on *Phragmites australis* to obtain the capacity of plant to respond nutrient availability. Two levels of P (low and high) and three levels of ammonia were used to grow *P. australis*. Nitrogen concentration was increased with nitrogen level in the root solution, but phosphorus level had no effect. The tissue concentration of phosphorus was affected by both N and P level, the effect of nitrogen being most pronounced. Nitrogen was reported as limiting nutrient for the growth of *P. australis* (Remore et al. 1999). Plant and substrate are major component of CW systems, substrate provides mechanical support to the plants, attachment site for microorganism to grow and remove pollutant through adsorption. Study has been done to compare the efficiency of gravel (A1 and A2) and soil (A3 and A4) based substrate with and without plant. Four CWs were constructed in bucket of same dimensions (60\*40\*60 cm), bucket was divided into three compartments (inlet, substrate, and outlet) plant used was *Pennisetum purpureum*. Ammonia removal was found higher in vegetated gravel based system (A1) than in vegetated soil based system

(A3). During initial period efficiency of A3 was found higher due to high HRT than A1 but found worse after 3 months of experiment (Yang et al 2001). A full scale constructed wetland system was developed to check the effectiveness of designing criteria (area of VFCW required per population equivalent) published by Danish government to treat sewage from a household with four persons. They have constructed a three chambered sedimentation tank of 2m<sup>3</sup> volume followed by VFCW of 15m<sup>2</sup> area planted with *Phragmites australis*, to enhance the performance of the system, half of its effluent was recirculated to the sedimentation tank in order to achieve denitrification. Phosphate was removed through filter media containing calcite and chemical precipitation in sedimentation tank. Phosphate removal from the wetland system was observed to be 20-30% if 3 m<sup>2</sup>/PE criteria used to treat waste water. Guideline published by Danish government for area requirement is effective for organic removal but for nutrient removal more study is required (Brix and Arias 2005). Danish criteria were found to be not suitable for phosphate removal and suggested further study for the same. Microbial activities were reported higher in A1 than A3. Phosphate removal was found higher in the A3 than A2 due to high adsorption capacity of soil. Modifications has been made to consider limitation of CWs like use of hybrid system or mixed culture wetlands etc. Vegetation used in CWs as mixed culture to overcome the limitation of each other. Mono culture and mixed culture of *S. validus* and *C. indica* was been studied to find their potential to remove N and P from effluent of secondary treatment. In the monoculture system above ground biomass of *S. validus* was higher however *C. indica* had higher below ground biomass. Growth of *S. validus* was found restricted in mixed culture. No significant increase in the removal efficiency was observed in he mixed culture than in mono culture (Zhang et al. 2007). CWs were studied to treat the eutrophic water of lake Taihu of south China. Three parallel scale CWs (VFS, HFS and FWS) were constructed of 30m<sup>2</sup> each used *T. angustifolia* plant. Ammonia removal was found higher in VSF than HFS and FWS systems (Li et al. 2007). Nitrate removal efficiency was 63%, 65%, and 34% in VSF, HSF, and FWS, respectively. Phosphate removal was found higher in VSF and HSF than FWS systems. Overall performance of VSF was found better than HSF and FWS to treat eutrophic water of lake Taihu. Efficiency of *Ipomoea aquatica* Forsk were detected to remove nutrients, chlorophyll, and COD from fishpond. Percentage removal of TN and TP was found 31%, and

19 %, respectively. The behaviour of plant in wetland with different concentration of nutrients in solution has been studied by many researchers in past few years. Changes in the nutrient characteristics of eutrophic water was examined with no additional culture substrate using the DFT (PVC trays of 4m long, 0.15m wide and 0.20m deep, regulated by a flow meter and an electric pump set at a rate of 60 L min) system and compared the efficiency of removal to a parallel system using *Ipomoea aquatica* Forsskal. Nutrients removal was tested using water without plant, water with plant, and Hoagland nutrient solution (having higher nutrients concentration than eutrophic pool water) with plant. Plant growth was found to be 1.5 times higher in nutrient solution than in pool water. Removal rate of effluent in planted system at 48hr was observed to be 84.5, 88.5, 91.1 and 68.8% for chemical oxygen demand, biochemical oxygen demand (BOD<sub>5</sub>), total suspended solids and Chlorophyll-a, respectively (Yang et al. (2008). It was observed that *I. aquatica* has high efficiency at temperature below 25°C (Li and Li 2009). Plants are major sink for nutrient in a constructed wetland but dead part of the plant can become a source if not harvested. Study of bioaccumulation of nutrients by *P. australis* in Albuñón rambla was done to observe the effect of cutting aerial biomass on nutrient bioaccumulation and water and sediment nutrient levels, and analysed seasonal variation of aerial biomass and nutrient concentrations (N and P) in *P. australis*, both in the aboveground and belowground tissues. Study was done between September 2003 and September 2004, this period includes two cutting events and two periods of reed growth, and the good climatic conditions in autumn favour their growth and another at the beginning of spring. Nine sampling points were selected in the floodplain, seven samplings were carried out during the annual cycle, and in each sampling, samples were taken of the aerial (stems, leaves, and panicles) and subterranean parts (roots and rhizomes) of common reed. Nitrogen and phosphorus concentration in the *P. australis* tissues was increased with N level in the root solution and significantly affected the relative growth rate of the plants (Ruiz and Velasco 2009). Seasonal changes in N and P concentrations in the aboveground and belowground tissues of common reed were observed in relation with plant phenology and cutting. The greatest nutrient concentrations were reached in the newly emerging stems after cutting. N concentrations in the aboveground reed tissues were found greater than in the belowground tissues, with maximum levels in growth periods (autumn and spring) and

minimum levels in winter and summer. Performance of wetlands is depended upon various physical parameters such as temperature, hydraulic retention time (HRT) etc. HRT has a direct impact on the size of a wetland system. Study was performed to monitor the impact of HRT on phosphate removal from CW. CW was found favourable to removal TP from waste water all year round. When the inflow TP concentrations were 0.87mg/l, the corresponding removal efficiency was measurement as 59.0% (Wu et al. 2009). Plant harvest had a critical contribution to the treatment of agricultural runoff by CW with a low P load. I was found that hydraulic retention time (1.7–3.8 d) had a significant correlation with the P removal efficiency, whereas water temperature, inflow P load, inflow, inflow P concentrations, and the hydraulic loading rate had a slight influence on P removal. High biomass growth was related with more nutrient uptake by plant and so high efficiency of wetland. Working of wetland is depended upon various physical factors such as, water hydrology, wetland type etc. A detailed study on working of different type of wetland was done to distinguish between them and to find order of efficiency of them. Mechanisms which are effective in the removal of contaminants from waste water were studied in different type of wetlands. FWS systems were said to be effective in removal of organics, basic mode of removal were microbial degradation and settling of colloidal particles, in addition to this HF and VF systems have filtration mechanism to removal solids (Vymazal 2010). Phosphate retention was stated to be low in HF and FWS. Denitrification is favourable in HF due to lack of oxygen and VF systems were found to be most effective in treating waste water but also have high operation and maintenance demand due to its pumping requirement. Many studies on constructed wetlands have focused on the effects of nutrient removal of wetland systems, in relation to wetland engineering design, operation mode and overall efficiency of nutrient removal by a few plant species. However, there have been very few studies on the species specific ecological characteristics and performances of plants used in constructed wetlands. As a representative of most commonly used plant species in Tai lake region of China, 15 plant species were investigated for their growth, morphological characteristics and nutrient uptake, with the aim of providing a scientific basis for selecting suitable plant species for constructed wetlands. The maximum plant above- and below-ground biomass was recorded for *Canna generalis* (15.5 and 6.1 g), *Thalia dealbata* (13.2 and 2.9 g), *Lythrum*

*salicaria* (12.8 and 4.6 g) and *Typha latifolia* (10.8 and 3.0 g). The total biomass of four plants was significantly higher than the other plants. Mean plant uptake accounted for 8.0–49.4% of  $\text{NH}_4^+$ -N, 17.8–59.6% of  $\text{NO}_3^-$ -N and 24.1–61.5% of P supplied to the culture solution. All 15 plant species showed significantly different nutrient uptake and storage rates under the same culture conditions. *C. generalis*, *T. latifolia*, *Th. dealbata* and *L. salicaria* had higher above- and below-ground biomass, nitrogen and phosphorus uptake than the other species (Jiang et al. 2011). Potential of a surface flow constructed wetland was found to treat polluted river in northern China. Four macrophyte species (*Typha orientalis*, *Phragmites australis*, *Scirpus validus* and *Iris pseudacorus*) were selected because they are very common in the area and also showed efficiency in the accumulation of nutrients and contaminants in constructed wetlands. Nutrient contents in above-ground and below-ground of all plants varied with time. The nutrient uptake by plants ranged from 14.29% to 51.89% of N removal, and accordingly 10.76% to 34.17% of P removal during the whole experiment. Furthermore, *S. validus* and *I. pseudacorus* had a higher nutrient uptake (Wu et al. 2011). On the whole, plant uptake is a significant removal process for nutrients. *S. validus* and *I. pseudacorus* are probably the preferred species in constructed wetlands for treating wastewater in northern China. Species and bioavailability of phosphorus in surface sediments from the middle and lower reaches of the Yellow River were investigated using the sequential extraction method. Sedimentary inorganic phosphorus was fractionated into four forms and the rank order according to the mean concentration of P-fraction in Yellow river. Twelve sediment samples (Y1-Y12) were collected from representative locations of the Yellow River from October to November in 2006. Sedimentary inorganic phosphorus in present study consists of four fractions (NaOH-P, BD-P,  $\text{NH}_4\text{Cl}$ -P and HCl-P). Inorganic phosphorus was the main content of phosphorus in Yellow River sediment as observed in the twelve sediments was present in the order of HCl-P > NaOH-P > BD-P >  $\text{NH}_4\text{Cl}$ -P (Wang et al. 2012). The linear relationship between the sum of BD-P and NaOH-P and the sum of active Fe and Al content is observed, which holds the assumption that active Fe and Al are the main sorbents for adsorbing P in natural sediments, BD-P and NaOH-P are mainly binding on Fe and Al (hydr)oxides. BAP are the sum of immediately available P and potentially available P. More than half of sedimentary inorganic phosphorus (mean 53%) were bio-available and can be conditionally released

into water column. Pilot scale constructed wetland was studied to treat the eutrophic water of Kolleru lake, Andhra Pradesh. The model was filled with the benthic soils, flora and fauna brought from the Kolleru Lake to mimic natural characteristics of the lake and a continuous flow was maintained using a control valve that allows the flow of water through a immediate reservoir. The flow rates of wastewater are controlled and the rate of flow was fixed approximately to 1 liter per hour. Based on the study, the artificial wetland can reduce the TDS, TSS, BOD, COD, nitrates and phosphates of the wastewater to a maximum extent in summer, followed by monsoon and winter season (Rao et al. 2013). It was observed that treatment efficiency was better during summer than winter season which was due to the increase in the bacterial growth and decomposition activities with increase in temperatures. The study concluded that, CW is a viable low cost alternative method of wastewater treatment for the treatment of the inflow wastewater joining the Kolleru lake resulting in the restoration of Kolleru lake water quality. *Canna indica* based VF constructed wetland has been studied to treat waste water under Indian conditions. A bench scale CW cell was constructed having dimensions of 1.1 m length  $\times$  0.8 m breadth  $\times$  0.35 m depth filled with sand gravel media. They have located their cell outdoor in a semi-arid climate without any protection from natural conditions. BOD and COD removal was observed to be effective with 87.3 and 92.8% removal, respectively, with COD:BOD ratio of 24.4. Growth of plant was observed to detect any water stress on it, and no direct stress was observed. Nutrients (Nitrogen as TKN and Phosphate) removal were also measured and found to be 82.6 and 89 % at 178.6 mg-P/m<sup>2</sup>-day and 852.3 mg-TKN/m<sup>2</sup>-day for phosphate and ammonia, respectively. They have concluded *Canna indica* as a suitable plant to use in constructed wetlands for phytoremediation under indian conditions (Haritash et al. (2015). Sediment can act as a source or sink of P under different environmental conditions. Inorganic P is the major form, and is a very useful indicator to evaluate the potential release of P in sediment. Study was done to evaluate the effects of pH and aerobic/anaerobic conditions on P release from sediment by the changes of P fractions before and after incubation. Sediment samples were collected from a drainage ditch of a livestock farm on reclaimed land in Kasaoka Bay, Japan. Experiments were conducted in a 200-mL glass serum bottle with 8 – 10 g fresh sediment and 100 mL of 0.02 M KCl solution, adjusted to pH 4, 7, or 10, and incubated under aerobic or anaerobic



conditions. Phosphorus was released under both aerobic and anaerobic conditions until day 5. The amount of P released under anaerobic conditions increased continuously towards day 10 while that under aerobic conditions decreased after day 5 to the end of experiment. Thus, sediment acted as a sink of P under aerobic conditions and as a source of P under anaerobic conditions (Nguyen and Maeda 2016). Sediment fractionations indicated that loosely sorbed P (loosely-P) and iron-bound P (Fe-P) fractions were the main sources of P released to the overlying water. The amount of P released from sediment at acidic pH was higher than that at neutral or alkaline pH under anaerobic conditions. However, under aerobic conditions, the amount of P release was higher at alkaline pH than that at acidic or neutral pH. Potential of *C. indica* towards phosphate removal was investigated in a pilot scale study, and quantified the distribution of phosphate in different plant tissues during phytoremediation. Average removal rate of TP (167 mg/m<sup>2</sup> day) was higher than that of AP (84 mg/m<sup>2</sup> day) as against the loading rate of 200 mg/m<sup>2</sup> day for TP and 85 mg/m<sup>2</sup> day for AP (Haritash et al. 2017). In plant, maximum increase of phosphate concentration was observed in leaves, followed by flowers, roots, and stem respectively. Study reported 40% more accumulation of P in shoots than roots at an influent level of 6.77 mg/l phosphate. Study concluded that the removal of P in *Canna indica*-based wetland is predominantly by plant uptake, and sediments too play a role in it.

## MATERIALS AND METHODS

Three pilot-scale vertical-flow constructed wetland cells vegetated with *Canna indica* (CW1), *Ipomoea indica* (CW2), and *Phragmites australis* (CW3) individually, are constructed in the campus of Delhi Technological University. The experiment work was started from June '18, July'18, and October'18 for *C. indica*, *I. indica* and *P. australis*, respectively, and had finished in March'19. Ambient temperature profile was taken from online source of Indian meteorological Department, to relate it with uptake of nutrients in different weather conditions. The CW cell has a packed media of 40-cm thick sand gravel substrate layer for *C. indica* and 40 cm thick gravel substrate layer for *I. indica*, in a cylindrical HDPE tank of diameter equal to 55 cm. CW cell for *P. australis* was packed with 90 cm thick gravel substrate layer in a cylindrical HDPE tank of 40 cm diameters. CW cell for *C. indica* was vegetated with total number of healthy stem count of 28 with average shoot height 120 cm, for *I. indica* it was 11 numbers of stem count with average shoot height of 70 cm, and average length of shoot of *P. australis* was 170 cm. The influent was fed from the top, and effluent was collected from an outlet placed at the bottom of tank above 10 cm from its base as shown in figure 1. The CW cell was initially flushed with water at a hydraulic loading rate (HLR) of 400 L/day for 5 days. Later, the plants were irrigated, on daily basis, with HLR of 30, 45 and 60 L/day of simulated wastewater for CW1 (*C. indica*), CW2 (*I. indica*), and CW3 (*P. australis*), respectively. Synthetic wastewater was prepared by dissolving Ammonium-dihydrogen-orthophosphate in groundwater, and it was fed all three CW cells on daily basis maintaining the hydraulic retention time (HRT) of 24 h on plug-flow basis. Inlet and outlet sample of the water was characterised for physic-chemical parameters such as pH, oxidation –reduction-potential (ORP), Temperature, and dissolve oxygen (DO). pH, ORP, temperature, and DO were measured in lab using Orion make Star A329 model multiparameter meter. Concentration of available phosphate (AP), and ammonical nitrogen were determined in influent and effluent of all three cells by using standard methods as prescribed by APHA (1995).

### **Ammonical nitrogen (Nesslerization method)**

Reagents: 1. Zinc sulphate solution - Dissolve 100 g of zinc sulphate  $ZnSO_4 \cdot H_2O$  and dilute to 1 litre with water. 2. Stabilizer reagent - Use EDTA or Rochelle salt to prevent calcium or magnesium precipitation in undistilled samples: a) EDTA reagent - Dissolve 50 g of EDTA in 60 ml water containing 10 g of sodium hydroxide. Heat gently to complete dissolution. Cool to room temperature and dilute to 100 ml. b) Rochelle salt solution - Dissolve 50 g of potassium sodium tartrate tetrahydrate in 100 ml of water. 3. Nessler's reagent - Dissolve 100 g of mercuric iodide and 70 g of potassium iodide in a small quantity of water and add this mixture slowly, with stirring, to a cool solution of 160 g of sodium hydroxide dissolved in 500 ml of water. Dilute to 1 litre.

Preparation of standards: 1. Stock ammonia solution - Dissolve 3.819 g of anhydrous ammonium chloride in water and dilute to 1 litre. 2. Permanent colour solutions a) Potassium chloroplatinate solution - Dissolve 2.0 g of potassium chloroplatinate in 300 to 400 ml of water, add 100 ml of concentrated hydrochloric acid and dilute to 1 litre. b) Cobaltous chloride solution - Dissolve of water. Add 100 ml of concentrated

Analysis of available ammonical nitrogen:

1. To 10 ml of sample add 1 ml of Nessler's reagent.
2. To this add 2 drops of EDTA solution in it.
3. Absorbance was noted at 425nm and 100% transmitted on spectrophotometer.
5. Multiplication of absorbance with graph factor gave the ammonia concentration of the sample.
6. Care should be take that absorbance is measured immediately after adding reagents.

### **Phosphate (By ammonium molybdate stannous chloride method)**

Reagents: 1. Ammonium Molybdate: In a flask, 25 g of ammonium molybdate was dissolved in 175ml of distilled water. In a separate conical flask 280ml of conc. Sulphuric acid in 400 ml of distilled water was added. The 2 solutions were mixed to make the final volume one litre. 2. Stannous chloride: For preparation, 2.5 g of stannous chloride was

dissolved in 100ml of glycerol. The solution was heated with intermittent mixing to make a clear viscous solution.

Preparation of standards: 0.143 g of potassium di- hydrogen phosphate was mixed in 1 litre of distilled water to obtain 100 ppm of phosphate stock standard solution. 10 ml of this solution was taken and final volume was made 100 ml to obtain 10 ppm of phosphate standard solution. Serial dilution was used to prepare 10 standards in the range of 0.1 to 1ppm. Distilled water was used as blank, which has zero phosphate concentration.

Preparation of the standard curve:

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

Analysis of available phosphate:

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

## **Phosphate in the sediments**

Initial and final phosphate content in the sediments of *C indica* substrate was measured using the SMT protocol as described by Ruban et al. (2001). Extraction was done to determine; NaOH extractable P (Fe/Al bound P or non-apatite P), HCl extractable P (Ca bound P or apatite P), and total P. SRP concentrations were analysed using the molybdenum blue method.

### **Sequential Extraction Procedure**

#### **NaOH-extractable P (NAIP) and HCl-extractable P (AIP)**

1. Weigh 200 mg of dry sediment in a centrifuge tube. It is important to keep the sediment/volume ratio constant. A 200 mg amount of sediment is the minimum required.
2. Add, with a pipette, 20 ml of 1 mol L<sup>-1</sup> NaOH.
3. Cover the tube and stir overnight (16 h). mixing is necessary; the sediment must be kept in suspension
4. Centrifuge at 2000 g for 15 min.

#### **NaOH-P (NAIP)**

1. Collect the extract.
2. Set apart (with a pipette) 10 ml of the extract in a test-tube.
3. Add 4 ml of 3.5 mol L<sup>-1</sup> HCl.
4. Stir energetically for 20 s and allow standing overnight (16 h). Cover the tube.
5. A brown precipitate appears and progressively settles. Centrifuge at 200 g for 15 min.
6. NaOH-P is measured on the supernatant.

#### **HCl-P (AIP)**

1. Wash the cake of the centrifugation in A.iv with 12 ml of 1 mol L<sup>-1</sup> NaCl. Stir for 5 min.
2. Centrifuge at 2000 g for 15 min and discard the supernatant.
3. Add with a pipette 20 ml of 1 mol L<sup>-1</sup> HCl.
4. Cover the tube and stir overnight (16 h).
5. Centrifuge at 2000 g for 15 min.
6. HCl-P is measured on the extract.

### **Concentrated HCl-extractable P (TP)**

1. Weigh 200 mg of dry sediment in a porcelain crucible.
2. Calcinate at 450 °C for 3 h.
3. Pour the cool ash into a centrifuge tube.
4. Add 20 ml of 3.5 mol L<sup>-1</sup> HCl with a pipette. HCl can be added directly to the crucible to ease the transfer of the ash.
5. Cover the tube and stir overnight (16 h).
6. Centrifuge at 2000 g for 15 min.
7. Collect the extract in a test-tube for the analysis of concentrated HCl-P.

### **Components of Constructed Wetlands cells**

In order to strengthen the integrated function of a wetland system, it is important to use suitable plant species and a highly cost-effective substrate. Plant richness can improve the structure, and function of wetland ecosystems to resist seasonal changes. Use of adsorbents can enhance the buffering capacity of the treatment cells to resist pollutant loads (Wu et al. 2018). Based on previous studies that documented ability of plants to survive in nutrient rich environment without any stress, rapid growth period and potential to treat waste water, the plant species that were used to establish a wetland plant community; *Phragmites australis* (common reed), *Canna indica*, and *Ipomoea indica*. Three individual wetland cells CW1, CW2, and CW3 were constructed and planted with *C. indica*, *I. indica*, and *P. australis* plants, respectively, with sand and gravel-packed supporting matrix as substrate in CW1 (*C. indica*) and gravel substrate in CW2 (*I. indica*) and CW3 (*P. australis*).

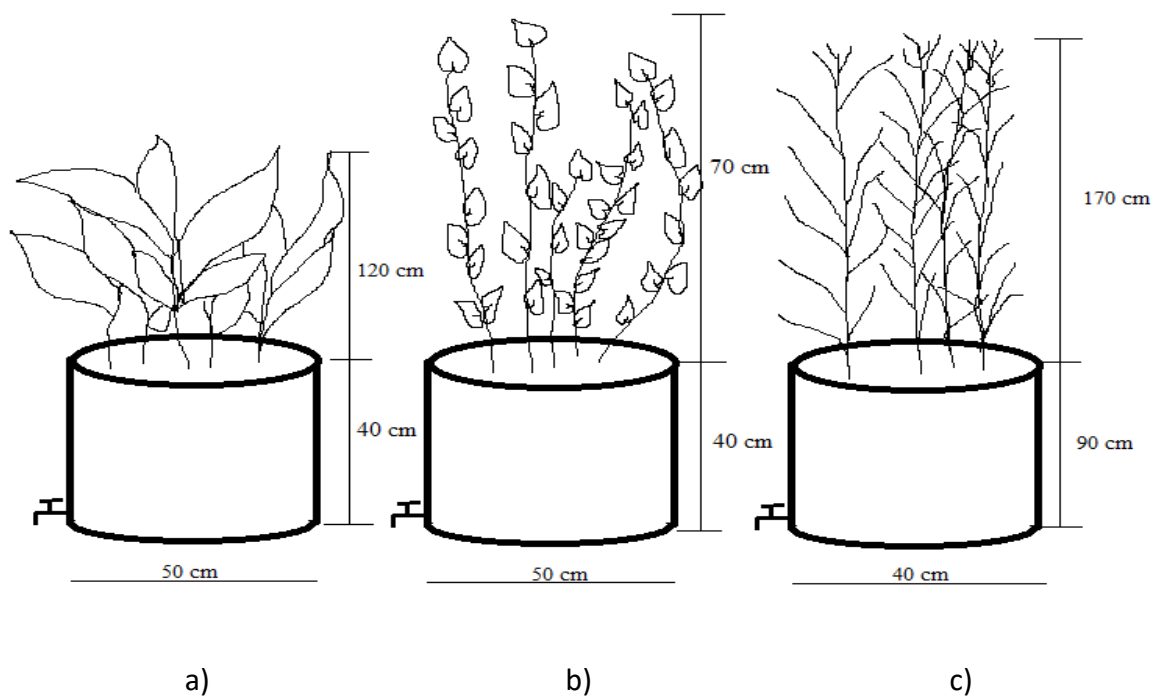


Fig. 1. Experimental setup of constructed wetland cells with a) *Canna indica*, b) *Ipomoea indica*, and c) *Phragmites australis* vegetation.

## RESULTS AND DISCUSSION

Parameters determining the water quality i.e. pH, ORP, and DO were measured during the study as monthly average to observe their role on removal and/or transformation of nutrients in wetland cells. Ambient mean monthly temperature was also been observed. Results of the water quality parameters as mean monthly value are shown in table 1. Average monthly pH of the inlet samples was observed as slightly basic (8.0 to 8.6) due to the presence of anions ( $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ ) and was suitable to convert  $\text{NH}_4^+$  into nitrate (7.5-8.6). Transformation of ammonia through volatilisation is a pH dependent process, as on pH (>9) ionic ammonia liberated into atmosphere as ammonia gas (Vymazal 2007). As pH of waste water in all three cells was below 9.0, volatilisation of ammonia was absent. Effective change in the pH was observed in outlets of all three cells, varied from 7.2 to 7.8, 7.6 to 8.1 and 7.2 to 8.1 in CW1 (*C. indica*), CW2 (*I. indica*), and CW3 (*P. australis*), respectively. Change in the pH from inlet to outlet was observed to be maximum for *C. indica* and minimum for *I. indica*. Adsorption of Ca-bound P and Fe/Al bound P is pH depended process, favourable pH was reported to be 8 to 10 for Ca bound P and <8 for Fe/Al bound P (Verhoeven and Meuleman 1999; Wang et al. 2005). In our study pH was observed to be favourable for adsorption of Fe/Al bound P (<8). DO is consumed in various bio-chemical processes to convert one form of nutrient into another, and also decides oxidation state of elements. Iron is present as  $\text{Fe}^{3+}$  in an aerobic system which binds phosphorous and gets removed through precipitation or adsorption. There will be no release of Fe/Al bound P is oxygen is present in the system (Nguyen and Maeda 2016). Waste water entered in cells was observed to be aerobic with inlet DO varied from 3.5 to 7.5 mg/l. DO present in waste water at inlet and DO that is released by plants roots are consumed in transformation of organics and nutrients. Significant change was observed in the concentration of DO in outlet of CW1 (*C. indica*), CW2 (*I. indica*), and CW3 (*P. australis*) samples varied from 0.9 to 3.8 mg/l, 2.3 to 5.8 mg/l and 1.8 to 5.5 mg/l, respectively. DO was observed to be minimum in the outlet of *C. indica* based cell which is due to the sand based substrate which cause root zone effect and keep soil oxygen deficient (Yang et al. 2001).

ORP of inlet water



Table 1: Water quality parameters (pH, ORP, and DO) in inlet and outlet of all three cells with ambient temperature.

		JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR
		(n=10)	(n=15)	(n=16)	(n=16)	(n=23)	(n=18)	(n=15)	(n=15)	(n=14)	(n=7)
pH	Inlet	8.3	8.0	8.2	8.5	8.6	8.2	8.3	8.0	8.0	8.0
	Outlet CW1	7.4	7.5	7.5	7.6	7.6	7.8	7.7	7.2	7.2	7.2
	Outlet CW2	-	8.2	8.1	8.1	8.2	7.9	8.1	7.6	7.7	7.8
	Outlet CW3	-	-	-	-	8.1	7.8	7.8	7.4	7.3	7.2
ORP (mV)	Inlet	165	166	173	114	127	158	159	183	53	48
	Outlet CW1	154	168	187	123	121	120	165	154	61	68
	Outlet CW2	-	165	188	122	118	119	163	152	66	85
	Outlet CW3	-	-	-	-	117	116	162	150	63	68
DO (mg/l)	Inlet	3.9	4.8	5.1	5.7	5.1	7.5	5.3	3.5	4.6	4.4
	Outlet CW1	0.9	1.7	2.1	2.0	2.0	3.2	3.0	2.2	3.6	3.8
	Outlet CW2	-	4.7	4.6	5.2	5.8	4.9	3.7	2.3	4.8	4.6
	Outlet CW3	-	-	-	-	4.9	4.0	5.5	1.8	5.2	3.2
Temperature (°C)	Min	30	28	26	25	20	15	8	8	11	12
	Max	40	37	35	33	34	28	22	20	24	25

samples was varied from 48 mV (March'19) to 183 mV(January'19), which indicates that waste water entered into all three cells was sufficiently oxic during the initial period (Jun'18 to Jan'19) and moved to anoxic during later phase (Feb'19-Mar'19). Redox potential in outlet samples of all three cells was found to be higher than that of inlet samples varied from 61 to 187 mV, 66 to 188 mV and 63 to 162 mV for CW1 (*C. indica*), CW2 (*I. indica*), and CW3 (*P. australis*), respectively. It is been reported by Moore and Reddy (1994) that no Fe/Al bound P is release above 0mV ORP at any pH between 5.5 and 8.5. Working of a constructed wetland also depends on physical parameter like temperature. Mineralisation of organically bound P and removal of nitrogen through denitrification are temperature dependent processes and optimum temperature for denitrification is 30 °C (Yang et al. 2008). In this study ambient temperature was varied widely with colder winter (8 °C) to hotter summer (40 °C), causing significant change on working of all three wetlands.

#### **Performance of wetland cell**

The effect of waste water characteristics, and change in weather condition on the efficiency of a wetland system to remove nutrients (phosphate and ammonia) from waste water was been observed. Operation period is divided into monthly interval and mean monthly value of temperature were taken to relate weather conditions. To obtain water characteristics pH, DO, and ORP were recorded, and were correlated with the removal of nutrients. Phosphate concentrations (as monthly average) at the inlet and outlet of all three cells were plotted with months to obtain monthly efficiency of system to remove nutrients. Also, Graphs for percentage removal were plotted with days, to see the trend of removal efficiency of phosphate and ammonia for all three cells.

#### **Available phosphate removal in CW1 (*Canna indica*)**

Percentage removal of mean monthly available phosphate was observed as 78%, 72%, 71%, 67%, 57%, 53%, 47%, 25%, 25%, and 45% during June, July, August, September, October, November, December, January, February, and March respectively, as shown in figure 2. Maximum removal efficiency of phosphate in *C. indica* based cell was observed during first three days (80-87%) of the experiment

work. During initial stages of working, plants and sediments activity is reported to be high (Haritash et al. 2017), which could be attributed to maximum removal of phosphate during initial days of our study. Trend of phosphate removal have negative slope from June to January, and a positive slope from February to March. Negative slope indicates a decrease in removal efficiency with months which can directly correlated with the mean monthly temperature. As the temperature is reducing from June to January (40 to 20 °C) removal efficiency is also reducing. Similar trend has also been observed in other studies (Wu et al. 2018). Decrease in removal efficiency could also be occurring due to increase in plant biomass (DeBusk et al. 1995). Significant change in removal efficiency was observed after first month, which was due to the saturation of adsorption of P on soil surface, similar observation had also been reported in other studies (Verhoeven and Meuleman 1999; Brix and Arias 2005; Vymazal 2007; Haritash et al. 2015) after that removal efficiency got saturated in July-August (72-71%).

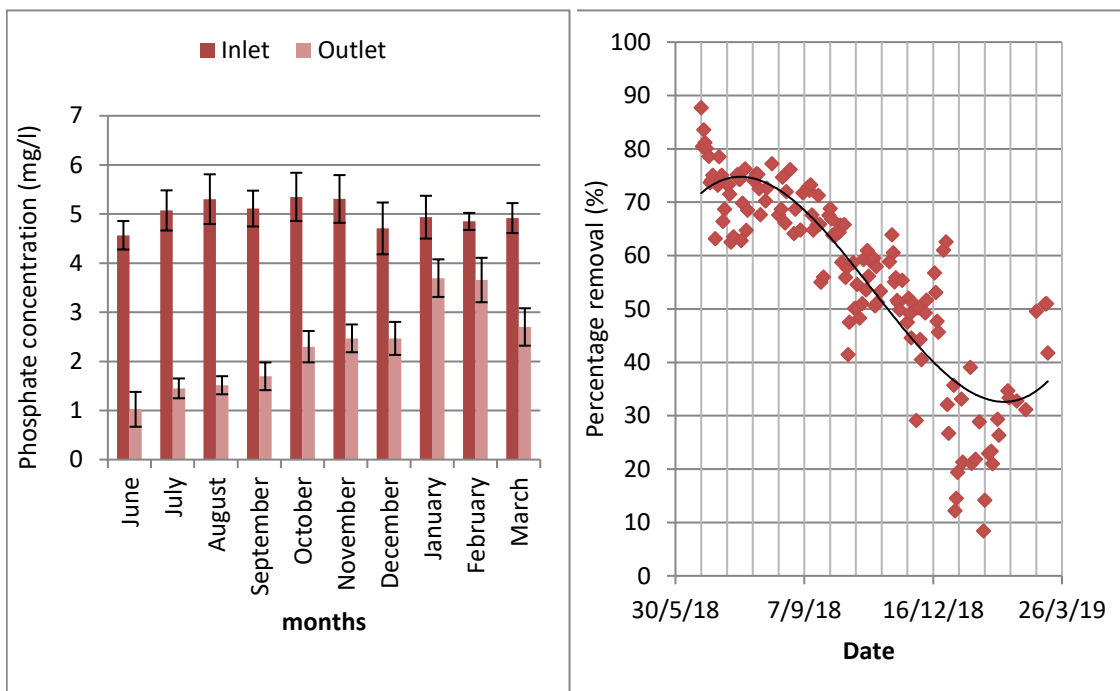


Fig. 2. Mean monthly variation of phosphate concentrations in inlet and outlet, and trend of percentage removal of phosphate in CW1 (*C. indica*).

### Total phosphate removal in CW1 (*Canna indica*)

Percentage removal of mean monthly total phosphate was observed as 71%, 66%, 62%, 59%, 54%, 44%, 48%, 31%, 40%, and 48% on June, July, August, September, October, November, December, January, February, and March respectively, as shown in figure 3. Removal of TP was higher in the initial growth phase of *C. indica*, and it reduces with time similarly to AP removal in *C. indica*, but with higher fluctuations and low percentage removal. Plants do not uptake OP directly, it first transform into AP by various bio-chemical processes and then utilises by plants. Transformation of bound phosphate to orthophosphate (AP) is majorly due to the microorganisms and enzymes secreted by plant roots (Haritash et al. 2017). Microbial activities are itself depended upon many factors and measurement of amount of P solubilized by microorganism is complex.

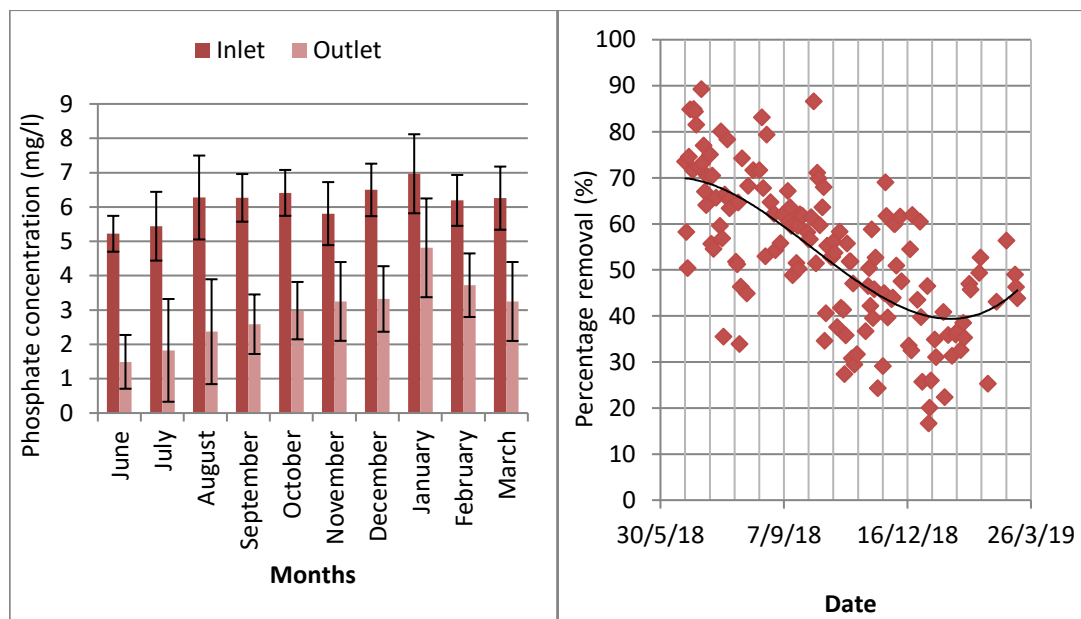


Fig. 3. Mean monthly variation of total phosphate concentrations in inlet and outlet, and trend of percentage removal of phosphate in CW1 (*C. indica*).

Table 2. Daily percentage removal of available phosphate (AP) and total phosphate (TP) in *C. indica* based cell (CW1)

Date	AP in IF	AP in OF	Percentage removal of AP	TP in IF	TP in OF	Percentage removal of TP
19-6-18	4.3	0.5	<b>87.7</b>	5.2	1.7	<b>67.2</b>
20-6-18	4.2	0.8	<b>80.5</b>	5.1	2.7	<b>46.9</b>

21-6-18	4.8	0.8	<b>83.6</b>	5.7	3.4	<b>41.7</b>
22-6-18	4.8	0.9	<b>81.2</b>	5.7	1.7	<b>70.8</b>
23-6-18	4.3	0.9	<b>80.1</b>	5.1	0.8	<b>84.7</b>
25-6-18	4.3	0.9	<b>78.6</b>	5.2	1.3	<b>75.2</b>
26-6-18	4.3	1.1	<b>73.7</b>	5.2	0.6	<b>89.0</b>
27-6-18	4.8	1.2	<b>74.1</b>	5.7	0.6	<b>90.1</b>
28-6-18	4.9	1.2	<b>75.1</b>	5.8	0.4	<b>94.0</b>
30-6-18	5.0	1.8	<b>63.2</b>	6.0	1.9	<b>67.8</b>
2-7-18	5.2	1.4	<b>73.2</b>	6.3	0.5	<b>91.3</b>
3-7-18	5.3	1.1	<b>78.6</b>	6.4	1.4	<b>78.1</b>
4-7-18	4.7	1.2	<b>74.4</b>	5.6	1.6	<b>71.6</b>
5-7-18	5.5	1.4	<b>75.1</b>	6.6	2.3	<b>65.9</b>
6-7-18	5.1	1.7	<b>66.4</b>	6.2	2.1	<b>66.6</b>
7-7-18	5.1	1.6	<b>68.7</b>	6.2	1.2	<b>80.3</b>
9-7-18	5.4	1.4	<b>73.4</b>	6.5	1.0	<b>85.2</b>
10-7-18	4.7	1.3	<b>73.2</b>	5.6	1.4	<b>75.5</b>
11-7-18	4.9	1.4	<b>71.5</b>	5.9	2.0	<b>66.7</b>
12-7-18	4.4	1.6	<b>62.5</b>	5.2	3.0	<b>43.5</b>
14-7-18	4.6	1.7	<b>63.6</b>	5.5	2.4	<b>55.6</b>
17-7-18	4.9	1.2	<b>75.2</b>	5.9	2.1	<b>65.1</b>
18-7-18	5.0	1.3	<b>74.4</b>	6.0	1.3	<b>79.1</b>
19-7-18	5.4	1.4	<b>74.3</b>	6.5	2.0	<b>68.9</b>
20-7-18	5.3	2.0	<b>62.8</b>	6.4	2.5	<b>60.0</b>
21-7-18	5.4	1.6	<b>69.8</b>	6.5	2.1	<b>68.1</b>
23-7-18	5.8	1.4	<b>76.3</b>	6.9	2.0	<b>71.5</b>
24-7-18	4.2	1.5	<b>64.8</b>	5.0	1.6	<b>68.4</b>
25-7-18	4.8	1.5	<b>68.5</b>	5.8	1.2	<b>78.5</b>
30-7-18	5.4	1.4	<b>74.1</b>	6.5	1.9	<b>69.9</b>
31-7-18	5.3	1.3	<b>74.8</b>	6.3	2.8	<b>55.3</b>
1-8-18	5.6	1.4	<b>75.4</b>	6.7	2.2	<b>66.6</b>
2-8-18	6.4	1.6	<b>75.2</b>	7.6	4.9	<b>36.0</b>
3-8-18	5.2	1.4	<b>72.5</b>	6.2	3.0	<b>51.6</b>
4-8-18	5.1	1.7	<b>67.7</b>	6.1	1.4	<b>77.4</b>
8-8-18	5.0	1.5	<b>70.3</b>	6.0	2.9	<b>50.5</b>
9-8-18	4.6	1.2	<b>72.7</b>	5.5	1.9	<b>66.0</b>
13-8-18	5.2	1.2	<b>77.2</b>	6.3	1.0	<b>83.7</b>
18-8-18	5.0	1.6	<b>67.7</b>	6.0	1.0	<b>82.8</b>
20-8-18	5.1	1.6	<b>68.7</b>	6.1	1.0	<b>83.1</b>
21-8-18	5.2	1.3	<b>74.7</b>	6.2	2.9	<b>52.9</b>
23-8-18	5.5	1.9	<b>66.1</b>	6.6	3.8	<b>42.5</b>
24-8-18	5.5	1.5	<b>72.0</b>	6.6	1.7	<b>74.1</b>
27-8-18	6.4	1.5	<b>76.1</b>	7.7	2.4	<b>69.0</b>
30-8-18	5.0	1.8	<b>64.2</b>	6.0	2.4	<b>59.6</b>
31-8-18	5.0	1.6	<b>68.7</b>	6.0	2.9	<b>51.1</b>
4-9-18	5.2	1.8	<b>64.8</b>	6.3	2.6	<b>59.0</b>

6-9-18	5.9	1.7	<b>71.8</b>	7.0	2.6	<b>63.3</b>
10-9-18	5.3	1.4	<b>72.8</b>	6.4	2.9	<b>53.7</b>
11-9-18	4.9	1.4	<b>72.2</b>	5.9	2.3	<b>61.3</b>
12-9-18	5.0	1.3	<b>73.2</b>	6.0	2.3	<b>62.3</b>
13-9-18	5.1	1.6	<b>67.6</b>	6.1	2.5	<b>58.2</b>
14-9-18	5.1	1.8	<b>64.8</b>	6.1	3.0	<b>51.1</b>
17-9-18	5.0	1.7	<b>65.8</b>	6.0	3.0	<b>50.1</b>
18-9-18	5.1	1.5	<b>71.3</b>	6.1	2.8	<b>53.9</b>
19-9-18	4.8	1.7	<b>65.9</b>	5.8	3.4	<b>40.7</b>
20-9-18	4.8	2.2	<b>55.1</b>	5.8	2.4	<b>58.8</b>
22-9-18	5.2	2.3	<b>56.0</b>	6.2	2.2	<b>65.2</b>
26-9-18	5.5	1.8	<b>67.6</b>	6.5	2.3	<b>64.9</b>
27-9-18	4.2	1.3	<b>68.8</b>	5.0	2.4	<b>52.7</b>
28-9-18	5.4	1.8	<b>66.9</b>	6.5	2.4	<b>63.2</b>
29-9-18	5.4	1.9	<b>63.9</b>	6.5	2.4	<b>63.2</b>
1-10-18	5.3	1.9	<b>63.8</b>	6.4	0.8	<b>87.0</b>
3-10-18	5.1	1.8	<b>64.7</b>	6.1	2.7	<b>54.9</b>
4-10-18	5.1	1.8	<b>64.4</b>	6.1	1.9	<b>68.5</b>
5-10-18	6.4	2.2	<b>65.7</b>	7.7	2.6	<b>66.0</b>
6-10-18	5.2	2.2	<b>58.7</b>	6.3	2.4	<b>61.3</b>
8-10-18	5.2	1.8	<b>65.9</b>	6.3	2.2	<b>65.1</b>
9-10-18	4.5	2.0	<b>55.9</b>	5.4	1.8	<b>66.0</b>
10-10-18	4.8	2.0	<b>57.7</b>	5.7	3.7	<b>34.9</b>
11-10-18	4.6	2.7	<b>41.4</b>	5.6	3.4	<b>39.1</b>
12-10-18	4.9	2.6	<b>47.6</b>	5.9	3.6	<b>38.9</b>
15-10-18	5.0	2.1	<b>58.7</b>	6.0	3.4	<b>44.1</b>
16-10-18	4.9	2.4	<b>50.1</b>	5.9	3.4	<b>42.8</b>
17-10-18	4.9	2.4	<b>50.1</b>	5.9	3.1	<b>46.8</b>
18-10-18	5.9	2.7	<b>54.6</b>	7.1	3.1	<b>56.2</b>
20-10-18	5.6	2.9	<b>48.3</b>	6.7	4.2	<b>37.1</b>
22-10-18	5.3	2.6	<b>51.0</b>	6.4	3.0	<b>53.6</b>
23-10-18	5.7	2.3	<b>59.3</b>	6.8	3.5	<b>48.7</b>
25-10-18	5.4	2.5	<b>53.6</b>	6.5	3.5	<b>46.5</b>
26-10-18	6.0	2.3	<b>61.0</b>	7.1	3.9	<b>44.8</b>
27-10-18	6.0	2.6	<b>56.2</b>	7.1	4.1	<b>43.1</b>
28-10-18	6.0	2.4	<b>59.2</b>	7.1	2.8	<b>60.8</b>
30-10-18	5.6	2.3	<b>59.0</b>	6.7	2.7	<b>60.5</b>
31-10-18	5.6	2.3	<b>59.6</b>	6.7	2.7	<b>60.5</b>
1-11-18	5.6	2.8	<b>50.6</b>	6.7	3.8	<b>43.2</b>
2-11-18	5.6	2.4	<b>57.9</b>	6.7	2.9	<b>56.5</b>
3-11-18	4.9	2.4	<b>51.2</b>	5.9	4.6	<b>21.9</b>
5-11-18	4.9	2.3	<b>53.3</b>	5.9	3.7	<b>36.9</b>
12-11-18	4.5	1.8	<b>58.9</b>	5.4	2.3	<b>57.0</b>
14-11-18	5.8	2.1	<b>63.9</b>	7.0	3.3	<b>53.1</b>
15-11-18	5.9	2.3	<b>60.6</b>	7.0	3.0	<b>57.2</b>

16-11-18	5.6	2.5	<b>55.1</b>	6.7	3.7	<b>44.9</b>
17-11-18	5.6	2.5	<b>55.9</b>	6.7	2.6	<b>60.7</b>
18-11-18	5.5	2.7	<b>51.6</b>	6.6	4.9	<b>26.6</b>
19-11-18	5.8	2.8	<b>51.2</b>	6.9	4.0	<b>42.2</b>
20-11-18	5.5	2.7	<b>49.9</b>	6.6	3.2	<b>51.8</b>
22-11-18	5.5	2.4	<b>55.4</b>	6.6	2.8	<b>57.9</b>
26-11-18	4.0	2.1	<b>47.6</b>	4.8	3.1	<b>35.3</b>
27-11-18	5.1	2.4	<b>52.0</b>	6.1	3.6	<b>40.9</b>
28-11-18	5.1	2.6	<b>49.1</b>	6.1	1.7	<b>72.6</b>
29-11-18	5.4	3.0	<b>44.6</b>	6.5	2.1	<b>68.1</b>
30-11-18	5.4	2.6	<b>51.3</b>	6.5	3.2	<b>49.7</b>
3-12-18	3.7	2.6	<b>29.1</b>	4.5	3.2	<b>27.2</b>
4-12-18	5.2	2.6	<b>50.2</b>	6.3	4.3	<b>31.2</b>
5-12-18	5.2	2.6	<b>50.2</b>	6.3	2.9	<b>53.2</b>
6-12-18	5.2	2.9	<b>44.3</b>	6.3	2.9	<b>53.2</b>
7-12-18	4.7	2.8	<b>40.5</b>	5.6	3.6	<b>36.1</b>
10-12-18	4.7	2.4	<b>49.3</b>	5.6	2.8	<b>50.0</b>
11-12-18	4.7	2.3	<b>51.6</b>	5.6	3.1	<b>44.7</b>
17-12-18	4.7	2.0	<b>56.7</b>	5.6	3.9	<b>29.9</b>
18-12-18	4.7	2.2	<b>53.1</b>	5.6	2.7	<b>52.0</b>
19-12-18	4.7	2.5	<b>47.7</b>	5.6	4.2	<b>24.9</b>
20-12-18	4.1	2.2	<b>45.6</b>	4.9	2.4	<b>51.0</b>
24-12-18	5.4	2.1	<b>61.0</b>	6.5	2.6	<b>59.7</b>
26-12-18	5.4	2.0	<b>62.6</b>	6.5	3.2	<b>50.9</b>
27-12-18	4.0	2.7	<b>32.1</b>	4.8	3.8	<b>20.8</b>
28-12-18	4.3	3.1	<b>26.7</b>	5.1	4.1	<b>20.5</b>
1-1-19	5.1	3.3	<b>35.7</b>	6.1	4.0	<b>34.6</b>
2-1-19	4.6	4.0	<b>12.2</b>	5.5	4.8	<b>11.6</b>
3-1-19	4.0	3.4	<b>14.5</b>	4.8	4.4	<b>8.1</b>
4-1-19	4.6	3.7	<b>19.4</b>	5.5	4.2	<b>23.3</b>
7-1-19	4.9	3.3	<b>33.2</b>	5.8	3.3	<b>43.6</b>
8-1-19	5.5	4.3	<b>21.3</b>	6.5	5.0	<b>23.0</b>
14-1-19	5.3	3.2	<b>39.1</b>	6.3	4.5	<b>28.4</b>
15-1-19	5.2	4.1	<b>21.1</b>	6.3	4.5	<b>27.7</b>
18-1-19	5.0	3.9	<b>21.9</b>	6.0	4.4	<b>26.1</b>
21-1-19	5.3	3.8	<b>28.9</b>	6.3	6.2	<b>2.4</b>
24-1-19	5.0	4.5	<b>8.4</b>	6.0	5.1	<b>14.2</b>
25-1-19	4.7	4.0	<b>14.2</b>	5.6	4.6	<b>18.7</b>
28-1-19	5.2	4.0	<b>23.0</b>	6.2	4.9	<b>22.3</b>
30-1-19	4.7	3.6	<b>23.4</b>	5.7	4.1	<b>27.7</b>
31-1-19	4.8	3.8	<b>21.0</b>	5.7	4.1	<b>28.9</b>
4-2-19	4.8	3.4	<b>29.4</b>	5.8	3.6	<b>38.4</b>
5-2-19	4.9	3.6	<b>26.4</b>	5.9	4.0	<b>33.2</b>
12-2-19	4.7	3.1	<b>34.7</b>	5.7	4.3	<b>25.1</b>
13-2-19	4.6	3.1	<b>33.4</b>	5.6	3.9	<b>30.3</b>

19-2-19	5.0	3.4	<b>32.8</b>	6.0	3.6	<b>40.5</b>
26-2-19	4.9	3.4	<b>31.2</b>	5.9	3.1	<b>47.4</b>
6-3-19	4.9	2.5	<b>49.6</b>	5.9	4.3	<b>27.6</b>
13-3-19	5.0	2.5	<b>51.0</b>	6.1	3.5	<b>41.9</b>
14-3-19	5.3	2.6	<b>51.0</b>	6.3	3.5	<b>45.5</b>
15-3-19	4.5	2.6	<b>41.8</b>	5.3	3.9	<b>27.2</b>

### Phosphate removal in CW2 (*Ipomoea indica*)

Percentage removal of mean monthly available phosphate and total phosphate was observed as 54%, 37%, 22%, 18%, 19%, 28%, 14%, 18%, and 20%, and 65%, 46%, 33%, 32%, 45%, 22%, 23%, 12%, and 27% during July, August, September, October, November, December, January, February, and March respectively, as shown in figure 4. Removal of TP was found higher than the AP in *I. indica* based cell, due to the presence of gravel based substrate which is more suitable of bacterial growth and high oxygen availability.

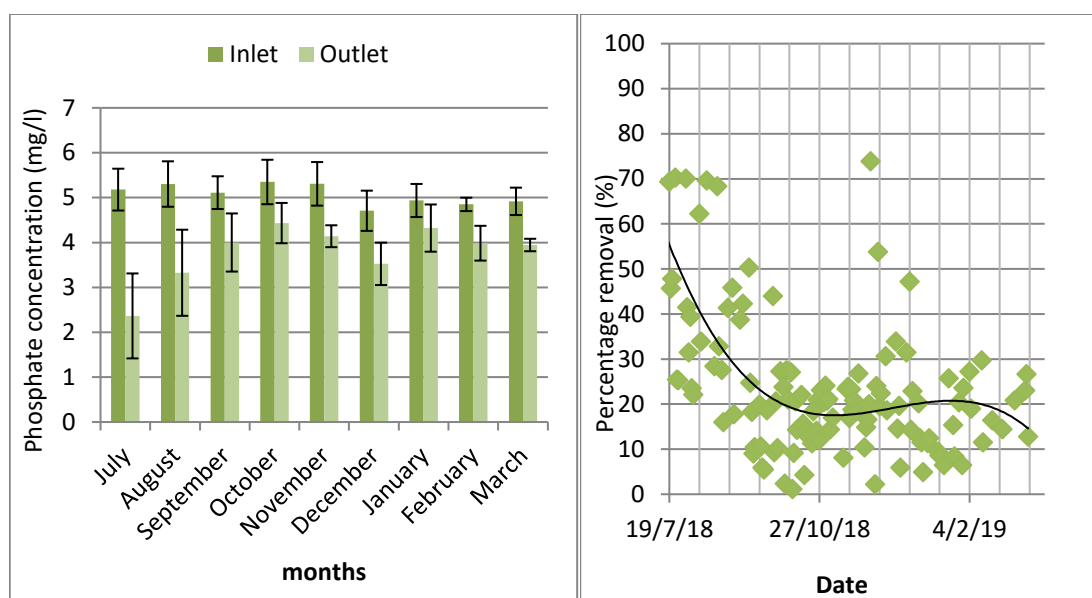


Fig. 4. Mean monthly variation of phosphate concentrations in inlet and outlet, and trend of percentage removal of phosphate in CW2 (*I. indica*).

Maximum removal efficiency of phosphate (57%) in *I. indica* based cell was observed during first month of experiment (July'18) due to high plant activity during initial days. Percentage removal reduced to 37%, 22%, and 18% during August'18, September'18 and October'18, respectively, due to low growth period as optimum



temperature for the growth of *I. indica* is stated 25 °C (Li and Li 2009) and temperature above and below this will lead to low nutrient removal efficiency. Effective increase in the biomass of plant was observed from mid of January'19 as average stem height got increased which could directly related to the increase in percentage removal during this period.

### Total phosphate removal in CW2 (*Ipomoea indica*)

Percentage removal of mean monthly total phosphate was observed as 65%, 46%, 33%, 32%, 45%, 22%, 23%, 12%, and 27% during July, August, September, October, November, December, January, February, and March respectively, as shown in figure 5. Removal of TP was found higher than AP in *I. indica*, as the microbial activities are higher under gravel based system (Haritash et al. 2015).

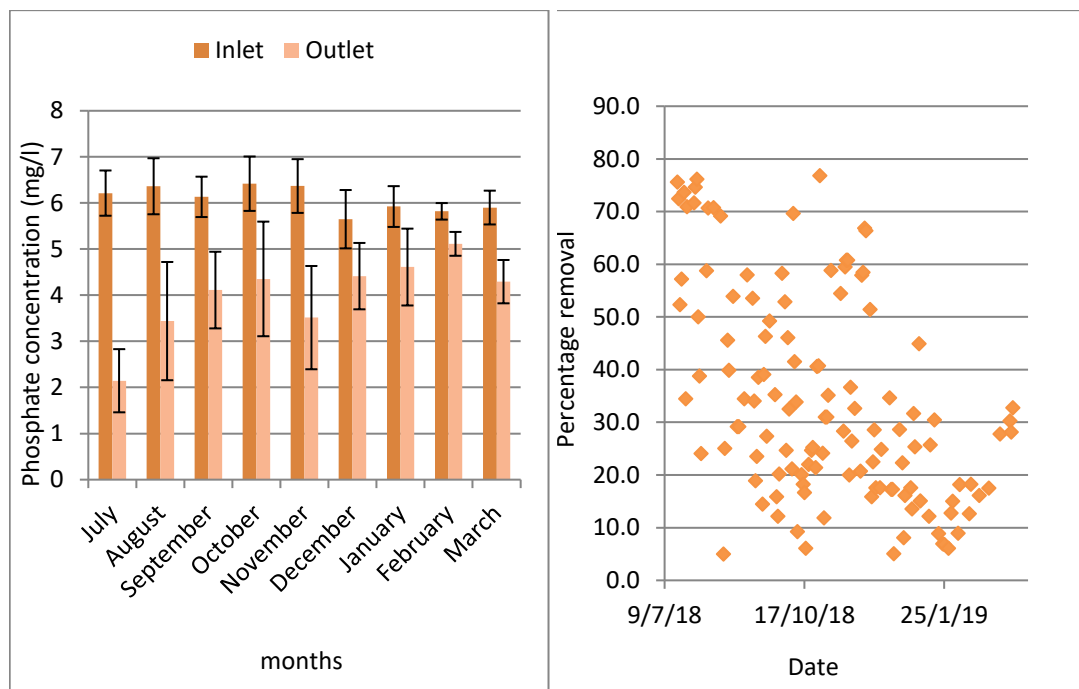


Fig. 5. Mean monthly variation of total phosphate concentrations in inlet and outlet, and trend of percentage removal of phosphate in CW2 (*I. indica*).

Table 3. Daily percentage removal of available phosphate (AP) and total phosphate (TP) in *I. indica* based cell (CW2)

Date	AP in IF	AP in OF	Percentage removal of AP	TP in IF	TP in OF	Percentage removal of TP
18-7-18	5.0	0.8	<b>84.8</b>	6.0	1.5	<b>75.6</b>

19-7-18	5.4	1.7	<b>69.3</b>	6.5	1.8	<b>72.5</b>
20-7-18	5.3	2.9	<b>45.7</b>	6.4	3.0	<b>52.3</b>
21-7-18	5.4	2.8	<b>47.8</b>	6.5	2.8	<b>57.2</b>
23-7-18	5.8	1.7	<b>70.2</b>	6.9	1.8	<b>73.6</b>
24-7-18	4.2	3.1	<b>25.4</b>	5.0	3.3	<b>34.5</b>
25-7-18	4.8	3.6	<b>25.3</b>	5.8	1.7	<b>70.9</b>
30-7-18	5.4	1.6	<b>70.0</b>	6.5	1.8	<b>71.7</b>
31-7-18	5.3	3.1	<b>41.5</b>	6.3	1.6	<b>74.6</b>
1-8-18	5.6	3.8	<b>31.4</b>	6.7	1.6	<b>76.2</b>
2-8-18	6.4	3.9	<b>39.4</b>	7.6	3.8	<b>50.0</b>
3-8-18	5.2	4.0	<b>23.5</b>	6.2	3.8	<b>38.8</b>
4-8-18	5.1	4.0	<b>22.1</b>	6.1	4.7	<b>24.1</b>
8-8-18	5.0	1.9	<b>62.2</b>	6.0	2.5	<b>58.8</b>
9-8-18	4.6	3.0	<b>33.9</b>	5.5	1.6	<b>70.7</b>
13-8-18	5.2	1.6	<b>69.6</b>	6.3	1.8	<b>70.7</b>
18-8-18	5.0	3.6	<b>28.4</b>	6.0	1.8	<b>69.2</b>
20-8-18	5.1	1.6	<b>68.3</b>	6.1	5.8	<b>5.0</b>
21-8-18	5.2	3.5	<b>32.8</b>	6.2	4.6	<b>25.0</b>
23-8-18	5.5	4.0	<b>27.6</b>	6.6	3.6	<b>45.6</b>
24-8-18	5.5	4.6	<b>16.0</b>	6.6	4.0	<b>39.9</b>
27-8-18	6.4	3.8	<b>41.3</b>	7.7	3.5	<b>53.9</b>
30-8-18	5.0	2.7	<b>45.8</b>	6.0	4.2	<b>29.2</b>
31-8-18	5.0	4.1	<b>17.7</b>	6.0	4.2	<b>29.2</b>
4-9-18	5.2	3.2	<b>38.7</b>	6.3	4.1	<b>34.4</b>
6-9-18	5.9	3.4	<b>42.2</b>	7.0	3.0	<b>58.0</b>
10-9-18	5.3	2.6	<b>50.3</b>	6.4	3.0	<b>53.5</b>
11-9-18	4.9	3.7	<b>24.7</b>	5.9	3.9	<b>34.0</b>
12-9-18	5.0	4.1	<b>18.3</b>	6.0	4.9	<b>18.9</b>
13-9-18	5.1	4.6	<b>9.0</b>	6.1	4.6	<b>23.5</b>
14-9-18	5.1	4.6	<b>10.5</b>	6.1	3.8	<b>38.5</b>
17-9-18	5.0	4.0	<b>19.9</b>	6.0	5.1	<b>14.5</b>
18-9-18	5.1	4.5	<b>10.5</b>	6.1	3.7	<b>39.1</b>
19-9-18	4.8	4.6	<b>5.9</b>	5.8	3.1	<b>46.3</b>
20-9-18	4.8	4.5	<b>5.5</b>	5.8	4.2	<b>27.4</b>
22-9-18	5.2	4.2	<b>18.6</b>	6.2	3.2	<b>49.2</b>
26-9-18	5.5	3.1	<b>44.0</b>	6.5	4.2	<b>35.3</b>
27-9-18	4.2	3.8	<b>9.3</b>	5.0	4.2	<b>15.9</b>
28-9-18	5.4	4.3	<b>20.6</b>	6.5	5.7	<b>12.1</b>
29-9-18	5.4	4.8	<b>10.2</b>	6.5	5.2	<b>20.2</b>
1-10-18	5.3	3.9	<b>27.3</b>	6.4	2.7	<b>58.3</b>
3-10-18	5.1	3.9	<b>23.8</b>	6.1	2.9	<b>52.9</b>
4-10-18	5.1	5.0	<b>2.4</b>	6.1	4.6	<b>24.7</b>
5-10-18	6.4	4.6	<b>27.5</b>	7.7	4.1	<b>46.1</b>
6-10-18	5.2	4.1	<b>21.0</b>	6.3	4.2	<b>32.5</b>
8-10-18	5.2	3.8	<b>27.1</b>	6.3	5.0	<b>21.2</b>

9-10-18	4.5	3.8	<b>14.6</b>	5.4	1.6	<b>69.6</b>
10-10-18	4.8	4.4	<b>9.2</b>	5.7	3.4	<b>41.5</b>
11-10-18	4.6	3.7	<b>20.6</b>	5.6	3.7	<b>33.8</b>
12-10-18	4.9	4.2	<b>14.3</b>	5.9	5.4	<b>9.2</b>
15-10-18	5.0	3.9	<b>22.0</b>	6.0	4.8	<b>20.1</b>
16-10-18	4.9	4.1	<b>15.6</b>	5.9	4.8	<b>18.3</b>
17-10-18	4.9	4.7	<b>4.2</b>	5.9	4.9	<b>16.6</b>
18-10-18	5.9	5.1	<b>13.8</b>	7.1	6.7	<b>6.1</b>
20-10-18	5.6	4.9	<b>12.7</b>	6.7	5.3	<b>22.0</b>
22-10-18	5.3	4.7	<b>11.3</b>	6.4	4.8	<b>24.7</b>
23-10-18	5.7	4.6	<b>18.4</b>	6.8	5.1	<b>25.2</b>
25-10-18	5.4	4.7	<b>14.0</b>	6.5	5.1	<b>21.4</b>
26-10-18	6.0	4.7	<b>20.9</b>	7.1	4.2	<b>40.6</b>
27-10-18	6.0	5.2	<b>12.1</b>	7.1	4.2	<b>40.7</b>
28-10-18	6.0	4.6	<b>23.1</b>	7.1	1.7	<b>76.8</b>
30-10-18	5.6	4.5	<b>20.5</b>	6.7	5.1	<b>24.1</b>
31-10-18	5.6	4.3	<b>24.1</b>	6.7	5.9	<b>11.9</b>
1-11-18	5.6	4.4	<b>20.8</b>	6.7	4.6	<b>31.0</b>
2-11-18	5.6	4.4	<b>21.1</b>	6.7	4.6	<b>31.0</b>
3-11-18	4.9	4.2	<b>14.3</b>	5.9	3.8	<b>35.1</b>
5-11-18	4.9	4.1	<b>16.9</b>	5.9	2.4	<b>58.8</b>
12-11-18	4.5	4.1	<b>8.1</b>	5.4	2.4	<b>54.4</b>
14-11-18	5.8	4.5	<b>23.3</b>	7.0	5.0	<b>28.4</b>
15-11-18	5.9	4.5	<b>23.8</b>	7.0	2.9	<b>59.5</b>
16-11-18	5.6	4.6	<b>16.9</b>	6.7	2.6	<b>60.7</b>
17-11-18	5.6	4.3	<b>23.3</b>	6.7	2.6	<b>60.7</b>
18-11-18	5.5	4.5	<b>18.8</b>	6.6	5.3	<b>20.0</b>
19-11-18	5.8	4.6	<b>20.8</b>	6.9	4.4	<b>36.6</b>
20-11-18	5.5	4.5	<b>18.3</b>	6.6	4.8	<b>26.4</b>
22-11-18	5.5	4.0	<b>26.8</b>	6.6	4.4	<b>32.7</b>
26-11-18	4.0	3.6	<b>10.4</b>	4.8	3.8	<b>20.7</b>
27-11-18	5.1	4.3	<b>14.9</b>	6.1	2.6	<b>57.9</b>
28-11-18	5.1	4.2	<b>16.6</b>	6.1	2.5	<b>58.5</b>
29-11-18	5.4	4.3	<b>19.9</b>	6.5	2.1	<b>66.9</b>
30-11-18	5.4	4.3	<b>19.9</b>	6.5	2.2	<b>66.4</b>
3-12-18	3.7	3.6	<b>2.2</b>	4.5	2.2	<b>51.4</b>
4-12-18	5.2	4.0	<b>24.0</b>	6.3	5.3	<b>15.9</b>
5-12-18	5.2	2.4	<b>53.8</b>	6.3	4.9	<b>22.5</b>
6-12-18	5.2	4.2	<b>19.6</b>	6.3	4.5	<b>28.6</b>
7-12-18	4.7	3.6	<b>22.3</b>	5.6	4.6	<b>17.6</b>
10-12-18	4.7	3.3	<b>30.6</b>	5.6	4.6	<b>17.6</b>
11-12-18	4.7	3.8	<b>18.6</b>	5.6	4.2	<b>24.9</b>
17-12-18	4.7	3.1	<b>33.9</b>	5.6	3.7	<b>34.6</b>
18-12-18	4.7	4.0	<b>14.6</b>	5.6	4.7	<b>17.3</b>
19-12-18	4.7	3.8	<b>19.6</b>	5.6	4.7	<b>17.3</b>

20-12-18	4.1	3.7	<b>8.8</b>	4.9	4.6	<b>5.0</b>
24-12-18	5.4	3.7	<b>31.5</b>	6.5	4.6	<b>28.7</b>
26-12-18	5.4	2.9	<b>47.2</b>	6.5	5.0	<b>22.3</b>
27-12-18	4.0	3.4	<b>14.2</b>	4.8	4.4	<b>8.1</b>
28-12-18	4.3	3.3	<b>22.8</b>	5.1	4.3	<b>16.1</b>
1-1-19	5.1	4.1	<b>20.2</b>	6.1	5.1	<b>17.6</b>
2-1-19	4.6	3.0	<b>34.4</b>	5.5	4.7	<b>13.6</b>
3-1-19	4.0	3.6	<b>11.5</b>	4.8	3.3	<b>31.6</b>
4-1-19	4.6	3.6	<b>22.0</b>	5.5	4.1	<b>25.4</b>
7-1-19	4.9	4.3	<b>11.4</b>	5.8	3.2	<b>44.9</b>
8-1-19	5.5	4.8	<b>12.5</b>	6.5	5.6	<b>15.1</b>
14-1-19	5.3	4.8	<b>9.4</b>	6.3	5.6	<b>12.2</b>
15-1-19	5.2	4.8	<b>8.6</b>	6.3	4.7	<b>25.7</b>
18-1-19	5.0	4.7	<b>6.4</b>	6.0	4.1	<b>30.5</b>
21-1-19	5.3	3.9	<b>25.7</b>	6.3	5.8	<b>8.9</b>
24-1-19	5.0	4.2	<b>15.3</b>	6.0	5.5	<b>6.9</b>
25-1-19	4.7	4.3	<b>8.4</b>	5.6	5.3	<b>6.8</b>
28-1-19	5.2	4.1	<b>20.4</b>	6.2	5.9	<b>6.1</b>
30-1-19	4.7	4.4	<b>6.5</b>	5.7	5.0	<b>12.7</b>
31-1-19	4.8	3.6	<b>23.6</b>	5.7	4.9	<b>15.0</b>
4-2-19	4.8	3.5	<b>27.2</b>	5.8	5.3	<b>8.9</b>
5-2-19	4.9	4.0	<b>19.0</b>	5.9	4.9	<b>18.2</b>
12-2-19	4.7	3.3	<b>29.7</b>	5.7	5.0	<b>12.6</b>
13-2-19	4.6	4.1	<b>11.5</b>	5.6	4.6	<b>18.3</b>
19-2-19	5.0	4.2	<b>16.4</b>	6.0	5.1	<b>16.1</b>
26-2-19	4.9	4.2	<b>14.4</b>	5.9	4.9	<b>17.5</b>
6-3-19	4.9	3.9	<b>20.8</b>	5.9	4.3	<b>27.8</b>
13-3-19	5.0	3.9	<b>23.0</b>	6.1	4.2	<b>30.3</b>
14-3-19	5.3	3.9	<b>26.6</b>	6.3	4.6	<b>28.2</b>
15-3-19	4.5	3.9	<b>12.8</b>	5.3	3.6	<b>32.8</b>

### **Phosphate removal in CW3 (*Phragmites australis*)**

Percentage removal of available phosphate was observed 96%, 85%, 61%, 31%, 33%, and 56% during October, November, December, January, February, and March, respectively. In the *P. australis* based cell, removal efficiency of phosphate was observed to be maximum (98%) on seventh day of the experiment work as shown in Figure 6, and was found fluctuating around this value for first fifteenth days. Percentage removal of phosphate was dropped to 61% as plant growth got restricted due to low temperature. *P. australis* has its senescent phase during winter, at this phase nutrient translocation gets reversed (from leave to roots) and nutrient uptake

gets limited (Ruiz and Velasco 2009). During senescent (January and February'19) removal rate was observed to be minimum i.e. 32%. Next growth phase was started at the end of February with significant increase in ambient temperature and percentage removal rate. Working of units in terms of phosphate removal had adversely affected by change in temperature in all three cells but *P. australis* based cells was found to be more efficient during all weather conditions. The worldwide availability of *P. australis* indicates its capacity to adapt different environment.

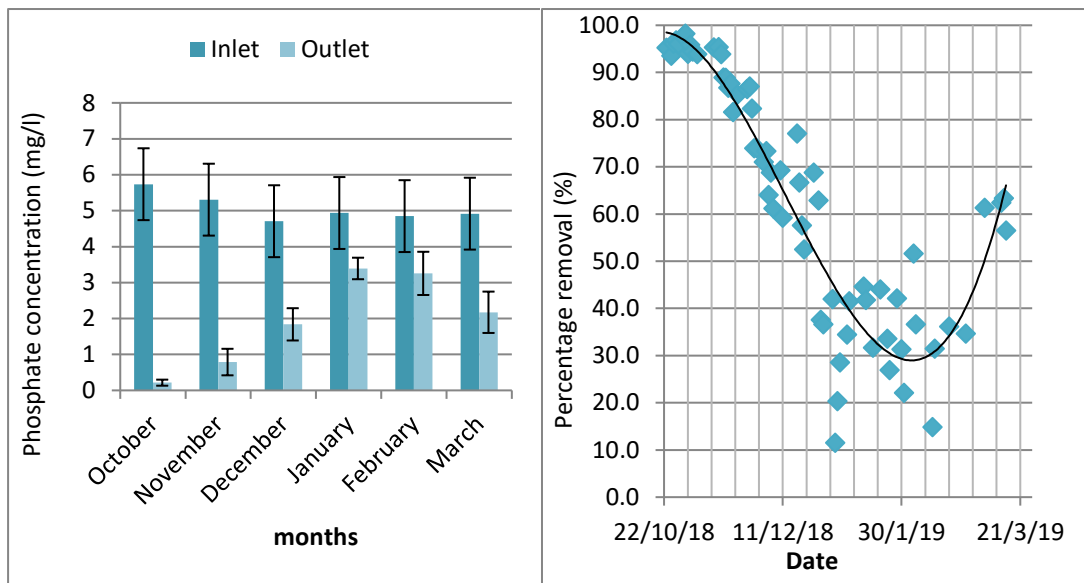


Fig. 6. Mean monthly variation of phosphate concentrations in inlet and outlet, and trend of percentage removal of phosphate in CW3 (*P. australis*).

#### Total phosphate removal in CW3 (*Phragmites australis*)

Percentage removal of mean monthly total phosphate was observed as 54%, 57%, 52%, 25%, 43%, and 46% on October, November, December, January, February, and March respectively, as shown in figure 7. Similar to *C. indica* TP removal in *P. australis* based cell was found to be lower than AP removal. *P. australis* was found less efficient for TP removal than *C. indica* for the months when DO of pore water was less in *P. australis* based wetland cell i.e. January and March'19.

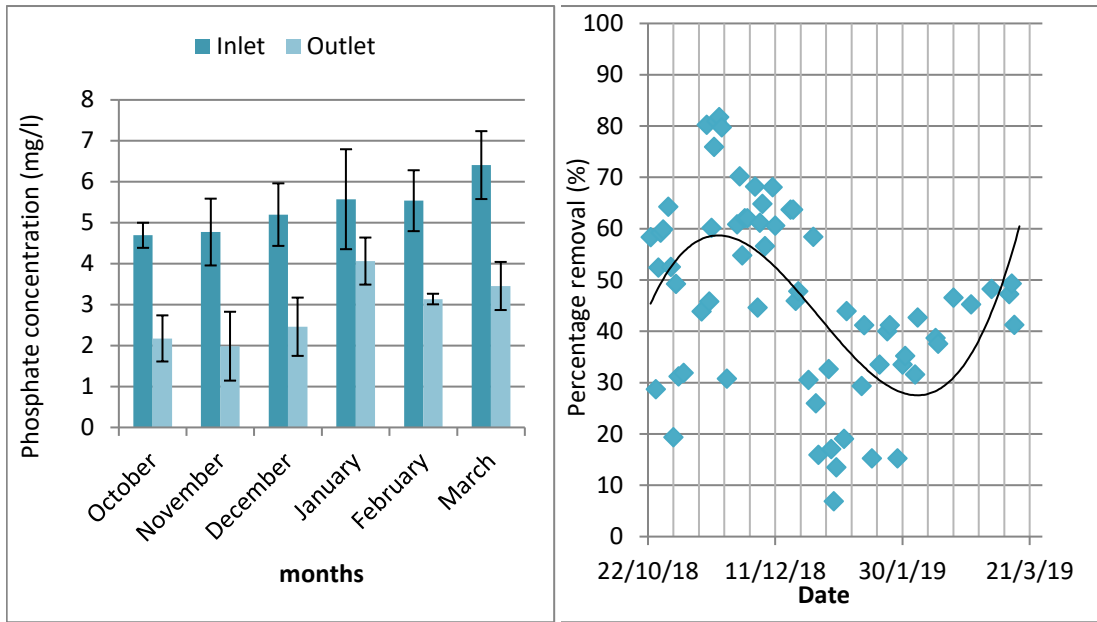


Fig. 7. Mean monthly variation of total phosphate concentrations in inlet and outlet, and trend of percentage removal of phosphate in CW3 (*P. australis*)

Table 4. Daily percentage removal of available phosphate (AP) and total phosphate (TP) in *P. australis* based cell (CW3)

Date	AP in IF	AP in OF	Percentage removal of AP	TP in IF	TP in OF	Percentage removal of TP
23-10-18	5.7	0.3	<b>95.2</b>	6.8	2.0	<b>70.7</b>
25-10-18	5.4	0.3	<b>93.5</b>	6.5	3.4	<b>48.0</b>
26-10-18	6.0	0.2	<b>96.2</b>	7.1	2.1	<b>71.1</b>
27-10-18	6.0	0.2	<b>96.8</b>	7.1	2.1	<b>71.1</b>
28-10-18	6.0	0.2	<b>96.1</b>	7.1	2.0	<b>71.5</b>
30-10-18	5.6	0.1	<b>97.6</b>	6.7	1.6	<b>76.5</b>
31-10-18	5.6	0.1	<b>98.2</b>	6.7	2.1	<b>68.8</b>
1-11-18	5.6	0.3	<b>94.0</b>	6.7	3.6	<b>47.0</b>
2-11-18	5.6	0.2	<b>95.9</b>	6.7	2.2	<b>66.7</b>
3-11-18	4.9	0.3	<b>94.4</b>	5.9	3.6	<b>39.0</b>
5-11-18	4.9	0.3	<b>93.9</b>	5.9	3.0	<b>49.7</b>
12-11-18	4.5	0.2	<b>95.3</b>	5.4	2.9	<b>45.7</b>
14-11-18	5.8	0.3	<b>95.4</b>	7.0	1.0	<b>86.1</b>
15-11-18	5.9	0.4	<b>93.9</b>	7.0	2.6	<b>62.6</b>
16-11-18	5.6	0.6	<b>88.9</b>	6.7	2.0	<b>69.6</b>
17-11-18	5.6	0.6	<b>88.9</b>	6.7	1.2	<b>81.7</b>
18-11-18	5.5	0.7	<b>86.7</b>	6.6	1.2	<b>81.5</b>
19-11-18	5.8	0.7	<b>87.7</b>	6.9	1.1	<b>84.4</b>
20-11-18	5.5	1.0	<b>81.6</b>	6.6	1.1	<b>83.5</b>
22-11-18	5.5	0.8	<b>85.3</b>	6.6	2.0	<b>69.2</b>

26-11-18	4.0	0.5	<b>86.5</b>	4.8	1.4	<b>71.4</b>
27-11-18	5.1	0.7	<b>87.0</b>	6.1	1.6	<b>74.4</b>
28-11-18	5.1	0.9	<b>82.4</b>	6.1	1.9	<b>68.1</b>
29-11-18	5.4	1.4	<b>74.0</b>	6.5	1.6	<b>74.7</b>
30-11-18	5.4	1.4	<b>74.0</b>	6.5	1.6	<b>74.7</b>
3-12-18	3.7	1.1	<b>71.0</b>	4.5	1.5	<b>67.2</b>
4-12-18	5.2	1.4	<b>73.4</b>	6.3	3.4	<b>45.6</b>
5-12-18	5.2	1.9	<b>64.0</b>	6.3	2.3	<b>63.7</b>
6-12-18	5.2	1.6	<b>68.8</b>	6.3	2.1	<b>67.1</b>
7-12-18	4.7	1.8	<b>61.2</b>	5.6	2.5	<b>54.9</b>
10-12-18	4.7	1.4	<b>69.3</b>	5.6	1.9	<b>66.8</b>
11-12-18	4.7	1.9	<b>59.2</b>	5.6	1.9	<b>66.8</b>
17-12-18	4.7	1.1	<b>77.1</b>	5.6	1.7	<b>69.4</b>
18-12-18	4.7	1.6	<b>66.7</b>	5.6	1.7	<b>69.4</b>
19-12-18	4.7	2.0	<b>57.6</b>	5.6	2.7	<b>51.9</b>
20-12-18	4.1	1.9	<b>52.5</b>	4.9	2.6	<b>46.4</b>
24-12-18	5.4	1.7	<b>68.8</b>	6.5	2.6	<b>60.4</b>
26-12-18	5.4	2.0	<b>62.9</b>	6.5	2.7	<b>58.7</b>
27-12-18	4.0	2.5	<b>37.6</b>	4.8	3.7	<b>22.2</b>
28-12-18	4.3	2.7	<b>36.6</b>	5.1	3.7	<b>28.0</b>
1-1-19	5.1	3.0	<b>42.0</b>	6.1	4.0	<b>34.2</b>
2-1-19	4.6	4.0	<b>11.5</b>	5.5	3.8	<b>29.7</b>
3-1-19	4.0	3.2	<b>20.3</b>	4.8	4.1	<b>14.4</b>
4-1-19	4.6	3.3	<b>28.5</b>	5.5	3.9	<b>28.3</b>
7-1-19	4.9	3.2	<b>34.5</b>	5.8	3.3	<b>43.9</b>
8-1-19	5.5	3.2	<b>41.5</b>	6.5	3.3	<b>49.9</b>
14-1-19	5.3	2.9	<b>44.6</b>	6.3	4.3	<b>31.6</b>
15-1-19	5.2	3.0	<b>41.8</b>	6.3	4.3	<b>30.9</b>
18-1-19	5.0	3.4	<b>31.7</b>	6.0	4.7	<b>21.8</b>
21-1-19	5.3	3.0	<b>44.0</b>	6.3	4.8	<b>24.5</b>
24-1-19	5.0	3.3	<b>33.6</b>	6.0	4.3	<b>27.4</b>
25-1-19	4.7	3.4	<b>27.0</b>	5.6	4.3	<b>23.4</b>
28-1-19	5.2	3.0	<b>42.1</b>	6.2	3.7	<b>41.3</b>
30-1-19	4.7	3.2	<b>31.4</b>	5.7	4.1	<b>27.7</b>
31-1-19	4.8	3.7	<b>22.1</b>	5.7	4.1	<b>28.2</b>
4-2-19	4.8	2.3	<b>51.6</b>	5.8	3.2	<b>44.6</b>
5-2-19	4.9	3.1	<b>36.6</b>	5.9	4.9	<b>18.0</b>
12-2-19	4.7	4.0	<b>14.9</b>	5.7	3.9	<b>31.5</b>
13-2-19	4.6	3.2	<b>31.5</b>	5.6	3.6	<b>35.5</b>
19-2-19	5.0	3.2	<b>36.2</b>	6.0	3.4	<b>43.5</b>
26-2-19	4.9	3.2	<b>34.7</b>	5.9	3.1	<b>47.3</b>
6-3-19	4.9	1.9	<b>61.3</b>	5.9	3.9	<b>33.9</b>
13-3-19	5.0	1.9	<b>62.4</b>	6.1	3.3	<b>46.1</b>
14-3-19	5.3	1.9	<b>63.4</b>	6.3	3.3	<b>48.8</b>
15-3-19	4.5	1.9	<b>56.5</b>	5.3	3.6	<b>32.6</b>

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### Nitrogen (as ammonia) removal in CW1 (*Canna indica*)

Plant uptake is a major source of nitrogen removal from wetlands but plant did not take ammonia directly. Nitrogen transformation in wetlands is influenced by temperature, pH, alkalinity, inorganic C source, moisture, microbial population, dissolved oxygen and concentration of ammonium-N (Vymazal 2007). Percentage removal of nitrogen as ammonia in *C. indica* based cell (CW1) was observed as 91%, 85%, 65%, 72%, and 74% during November, December, January, February, and March, respectively, as shown in figure 8. Ammonia removal was affected by change in temperature maximum release was found during June'18 when the ambient temperature was maximum (35°C) that agrees with Cooper et al. (1996) who observed that biological oxidation of ammonia is affected by temperature, optimum temperature for nitrification in soil is 30-40 °C and Hu et al. (2010) who stated that with increase in temperature ammonia release increases. Nitrogen removal was found maximum in the initial phase of growth of the plant and reduces as it approaches to maturity. Similar observation was found by Zhang (2007). Minimum removal of ammonia was observed during winter as ambient temperature fall minimum, similar observation was found by Jiang et al. (2008).

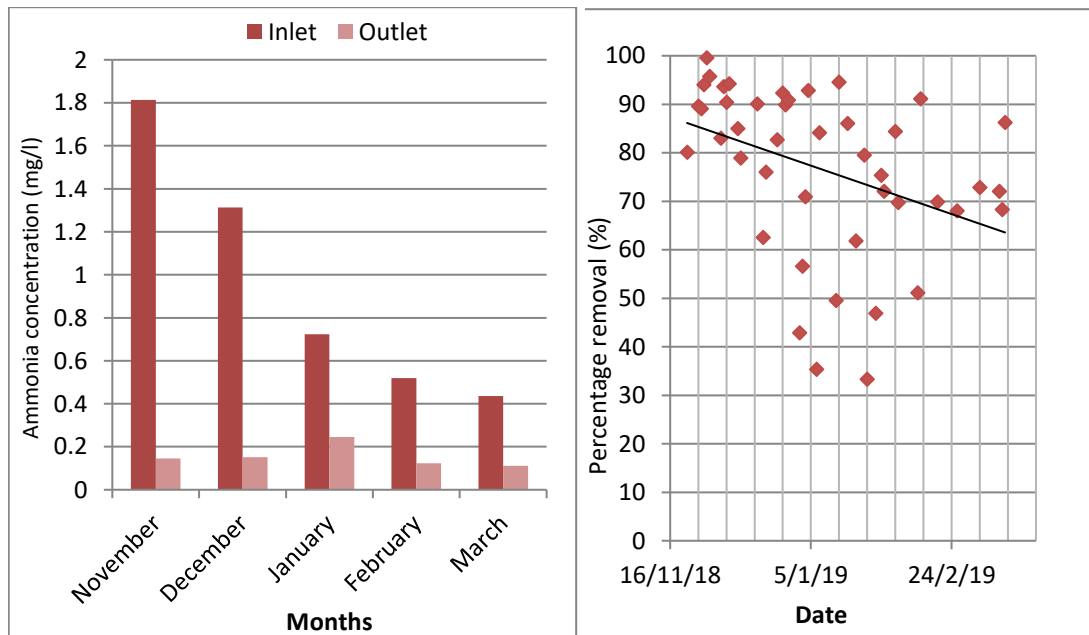


Fig. 8. Mean monthly variation of ammonia concentrations in inlet and outlet, and trend of percentage removal of ammonia in CW1 (*C. indica*).



Soil substrate used in CW1 has disadvantage of getting clogged which cause reduction in its porosity leads to low oxygen dissolved (Brix 1993). Reduction in oxygen will cause limited nitrification which causes reduction in removal rate with time. As the removal of ammonia by plant uptake increase with increase in inlet concentration (Comin et al. 1999; Zhang et al. 2007; Wu et al. 2018), maximum removal rate was observed at the inlet loading 227 mg-N/m<sup>2</sup>-day in all three cells.

### Nitrogen (as ammonia) removal in CW2 (*Ipomoea indica*)

Percentage removal of nitrogen as ammonia in in *I. indica* based cell (CW2) was observed as 94%, 83%, 81%, 85%, and 83% during November, December, January, February, and March, respectively, as shown in figure 9. Removal of ammonia was found to be higher in CW2 (*I. indica*) than that of CW1 (*C. indica*) as amounts of microorganisms growing on the media surface and plant roots were larger in the gravel-bed than those in the soil bed (Brix 1993; Yang et al. 2001). Growth of algae on the surface of substrate was also observed which caused volatilisation of ammonia due to increase in pH (>7.5).

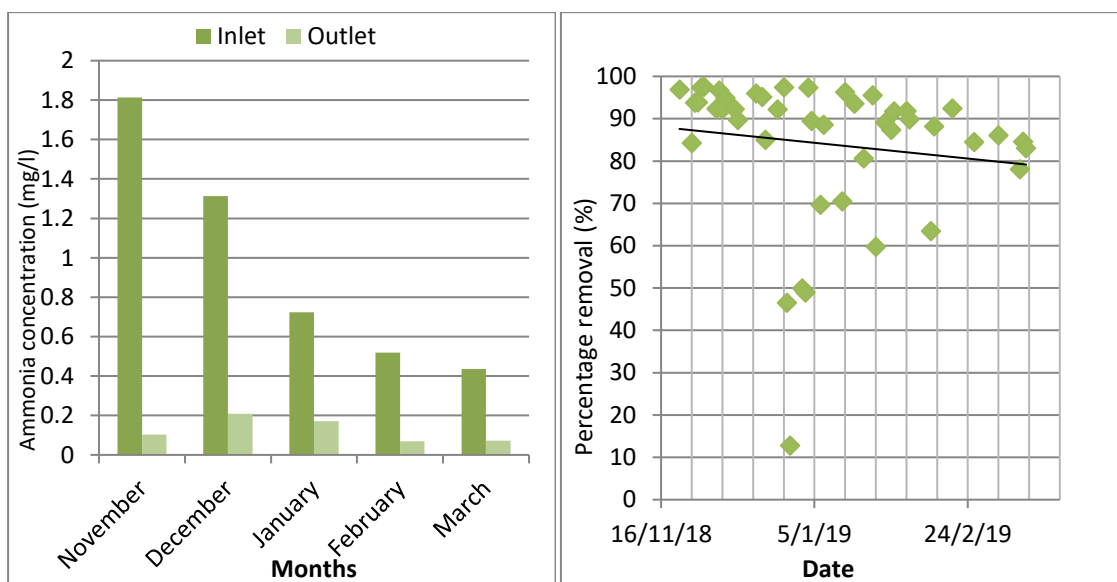


Fig. 9. Mean monthly variation of ammonia concentrations in inlet and outlet, and trend of percentage removal of ammonia in CW2 (*I. indica*).

### Nitrogen (as ammonia) removal in CW3 (*Phragmites australis*)

Percentage removal of nitrogen as ammonia in *P. australis* based cell (CW3) was observed as 97%, 76%, 70%, 78%, and 83% during November, December, January, February, and March, respectively, as shown in figure 10. Similar to *C. indica* and *I. indica* based cell nitrogen (as ammonia) removal in *P. australis* based cell was found maximum during initial phase and tapers off with time. Due to the presence of water root in *P. australis* which directly take nutrient from water removal of ammonia in *P. australis* based cell was found higher than both *C. indica* and *I. indica* based cell.

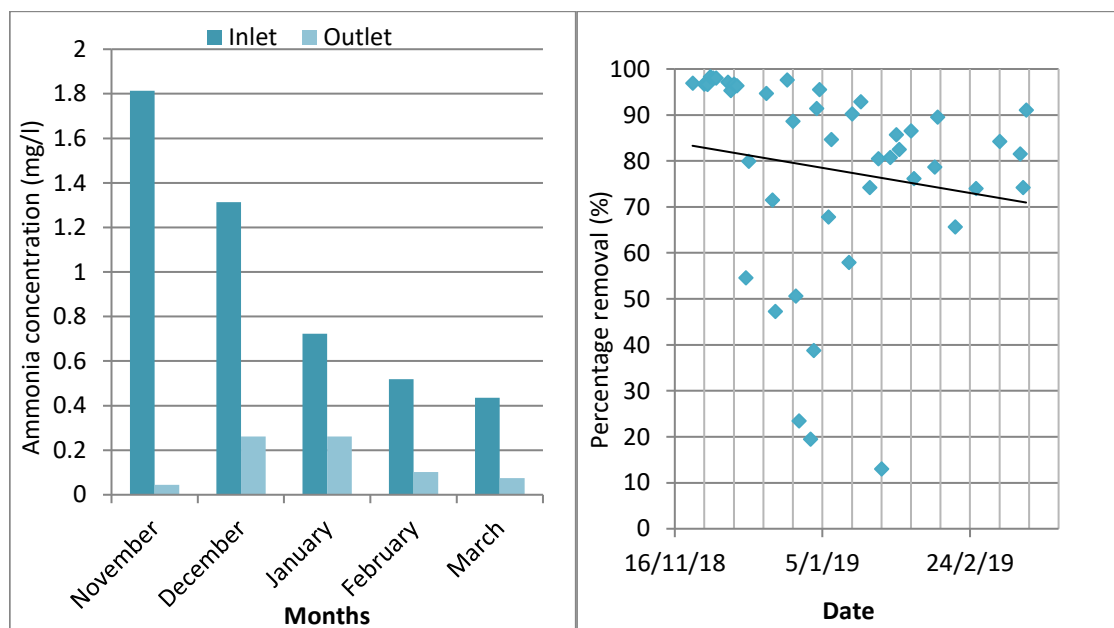


Fig. 10. Mean monthly variation of ammonia concentrations in inlet and outlet, and trend of percentage removal of ammonia in CW3 (*P. australis*).

Table 5. Daily percentage removal of ammonia in *C. indica*, *I. indica* and *P. australis* based wetland.

Date	Ammonia in IF	Ammonia in OF of CW1	%removal of ammonia in CW1	Ammonia in OF of CW2	%removal of ammonia in CW2	Ammonia in OF of CW3	%removal of ammonia in CW3
22-11-18	1.50	0.30	<b>80.10</b>	0.05	<b>96.93</b>	0.05	<b>96.93</b>
26-11-18	1.39	0.15	<b>89.56</b>	0.22	<b>84.27</b>	0.05	<b>96.70</b>
27-11-18	1.75	0.19	<b>89.06</b>	0.11	<b>93.77</b>	0.06	<b>96.63</b>
28-11-18	2.40	0.14	<b>94.00</b>	0.15	<b>93.87</b>	0.04	<b>98.36</b>
29-11-18	1.92	0.01	<b>99.54</b>	0.05	<b>97.39</b>	0.04	<b>98.08</b>
30-11-18	1.92	0.08	<b>95.74</b>	0.05	<b>97.60</b>	0.04	<b>98.08</b>

4-12-18	0.62	0.11	<b>82.96</b>	0.05	<b>92.38</b>	0.02	<b>97.11</b>
5-12-18	2.83	0.18	<b>93.61</b>	0.09	<b>96.67</b>	0.13	<b>95.33</b>
6-12-18	1.75	0.17	<b>90.35</b>	0.14	<b>92.26</b>	0.06	<b>96.70</b>
7-12-18	1.85	0.11	<b>94.20</b>	0.09	<b>94.94</b>	0.07	<b>96.37</b>
10-12-18	1.20	0.18	<b>84.96</b>	0.09	<b>92.32</b>	0.55	<b>54.58</b>
11-12-18	1.20	0.25	<b>78.87</b>	0.12	<b>89.76</b>	0.24	<b>79.97</b>
17-12-18	2.16	0.21	<b>90.06</b>	0.09	<b>96.03</b>	0.11	<b>94.73</b>
19-12-18	0.50	0.19	<b>62.50</b>	0.02	<b>95.17</b>	0.14	<b>71.55</b>
20-12-18	0.50	0.12	<b>75.98</b>	0.08	<b>85.06</b>	0.26	<b>47.29</b>
24-12-18	0.61	0.11	<b>82.68</b>	0.05	<b>92.25</b>	0.01	<b>97.62</b>
26-12-18	1.24	0.10	<b>92.31</b>	0.03	<b>97.44</b>	0.14	<b>88.66</b>
27-12-18	1.24	0.13	<b>89.82</b>	0.66	<b>46.50</b>	0.61	<b>50.65</b>
28-12-18	1.37	0.13	<b>90.84</b>	1.20	<b>12.80</b>	1.05	<b>23.49</b>
1-1-19	1.67	0.95	<b>42.87</b>	0.84	<b>49.95</b>	1.34	<b>19.53</b>
2-1-19	1.50	0.65	<b>56.57</b>	0.77	<b>48.93</b>	0.92	<b>38.80</b>
3-1-19	0.35	0.10	<b>70.92</b>	0.01	<b>97.37</b>	0.03	<b>91.45</b>
4-1-19	0.45	0.03	<b>92.79</b>	0.05	<b>89.53</b>	0.02	<b>95.54</b>
7-1-19	0.28	0.18	<b>35.32</b>	0.08	<b>69.67</b>	0.09	<b>67.86</b>
8-1-19	0.65	0.10	<b>84.09</b>	0.07	<b>88.57</b>	0.10	<b>84.69</b>
14-1-19	0.25	0.13	<b>49.54</b>	0.07	<b>70.51</b>	0.11	<b>57.95</b>
15-1-19	1.06	0.06	<b>94.55</b>	0.04	<b>96.27</b>	0.10	<b>90.27</b>
18-1-19	1.26	0.18	<b>86.01</b>	0.08	<b>93.60</b>	0.09	<b>92.89</b>
21-1-19	0.37	0.14	<b>61.79</b>	0.07	<b>80.68</b>	0.10	<b>74.27</b>
24-1-19	0.54	0.11	<b>79.47</b>	0.02	<b>95.52</b>	0.10	<b>80.50</b>
25-1-19	0.72	0.48	<b>33.27</b>	0.29	<b>59.81</b>	0.62	<b>13.01</b>
28-1-19	0.41	0.22	<b>46.87</b>	0.04	<b>89.09</b>	0.08	<b>80.76</b>
30-1-19	0.68	0.17	<b>75.34</b>	0.09	<b>87.38</b>	0.10	<b>85.74</b>
31-1-19	0.68	0.19	<b>72.02</b>	0.06	<b>91.76</b>	0.12	<b>82.55</b>
4-2-19	0.68	0.11	<b>84.37</b>	0.06	<b>91.84</b>	0.09	<b>86.59</b>
5-2-19	0.44	0.13	<b>69.71</b>	0.04	<b>89.85</b>	0.10	<b>76.18</b>
12-2-19	0.34	0.17	<b>51.10</b>	0.13	<b>63.49</b>	0.07	<b>78.68</b>
13-2-19	0.80	0.07	<b>91.09</b>	0.10	<b>88.18</b>	0.08	<b>89.58</b>
19-2-19	0.48	0.14	<b>69.84</b>	0.04	<b>92.41</b>	0.16	<b>65.70</b>
26-2-19	0.37	0.12	<b>68.02</b>	0.06	<b>84.48</b>	0.10	<b>74.02</b>
6-3-19	0.61	0.17	<b>72.80</b>	0.09	<b>86.07</b>	0.10	<b>84.27</b>
13-3-19	0.38	0.11	<b>72.03</b>	0.08	<b>78.07</b>	0.07	<b>81.53</b>
14-3-19	0.38	0.12	<b>68.25</b>	0.06	<b>84.59</b>	0.10	<b>74.21</b>
15-3-19	0.38	0.05	<b>86.24</b>	0.06	<b>83.10</b>	0.03	<b>91.11</b>

### Phosphate in plant tissues

Phosphate uptake by plant tissue is directly related to plant biomass and root oxidising capacity. A comparison of above ground and below ground biomass has been done by Jiang et al. (2011), and stated that plant accumulate more nutrient in

their above ground biomass than their below ground biomass. The contribution of plants to the nutrient removal is often only temporary because of loss of nutrients at senescence, followed by leaching and decomposition of litter (Verhoeven and Van der Toorn 1990). Nutrients can export from a system through regular harvesting of above ground tissue of plants, whereas autochthonous addition may result in recirculation (Haritash et al. 2017). Phosphate uptake by leaf, stem and flower of *C. indica*, and leaf and stem of *I. indica* and *P. australis* has been measured before and after the experimental work as shown in figure 8. Phosphate concentration during initial and final stage of experiment in the tissue of stem, flower and leaf of *C. indica* was observed as 13.3, 0.3, and 3.7 mg-P/g and 23.5, 9.3, and 7.5 mg-P/g, respectively. Maximum accumulation of nutrient in *C. indica* was observed in tissue of stem. If relative fraction of initial and final phosphorus concentration is compared, flowers accumulated more concentration of phosphate also been reported by Haritash et al. (2017).

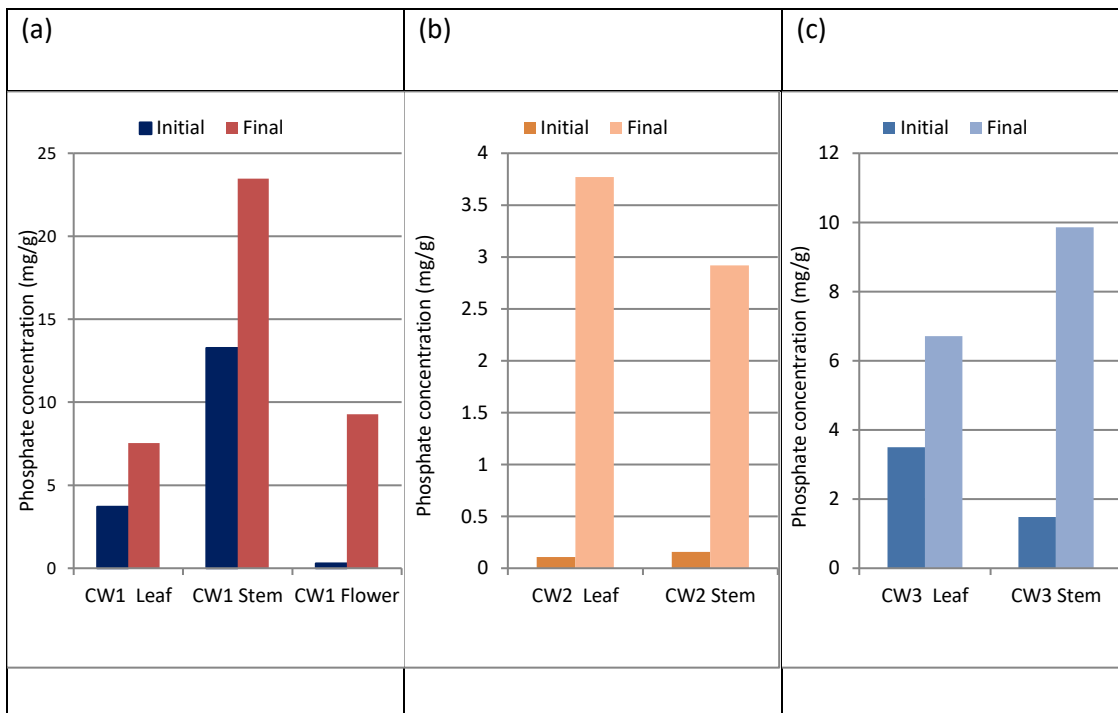


Fig. 11 Concentration of phosphate (mg-P/g) in different plant tissues of (a) *C.indica*,(b) *I. indica* and (c) *P. australis* during initial and final stage of treatment.

Initial and final concentration of phosphate in the tissue of leaf of *I. indica* and *P. australis* was observed as 0.1 and 3.7 mg-P/g, and 3.5, and 6.7 mg-P/g, and in stem

was 0.1, and 2.9 mg-P/g, and 1.5, and 9.8 mg-P/g, respectively. Accumulation of phosphate by *I. indica* was found minimum due to low growth of the plant at available temperature. Accumulation of phosphate in the tissue of *P. australis* was significant. Phosphate accumulation in tissue of stem was found higher than in leaf, similar observation has been reported by Comin et al. (1999).

### Phosphate in plant tissue of *Canna indica*

Plant can act as source of nutrients if dead tissue is not exported from a wetland system (Haritash et al. 2015). Although nutrient exported through harvesting is insignificant as compared to nutrient loading in waste water but it will contribute a significant amount of nutrient after decomposition into the system (Brix 1993; Verhoeven and Meuleman 1999; Jiang et al. 2008; Hu et al. 2010), so it become important to analyse how much load will going to be add due to plant litter. *C. indica* has distinct characteristic of growth cycle, due to which it produces more litter than *P. australis* and *I. indica*. During the study four growth cycles were observed and dry part of *C. indica* was harvested during all cycles. Harvesting was done after 72, 128, 150 and 179 days of start (18<sup>th</sup> June 2018).

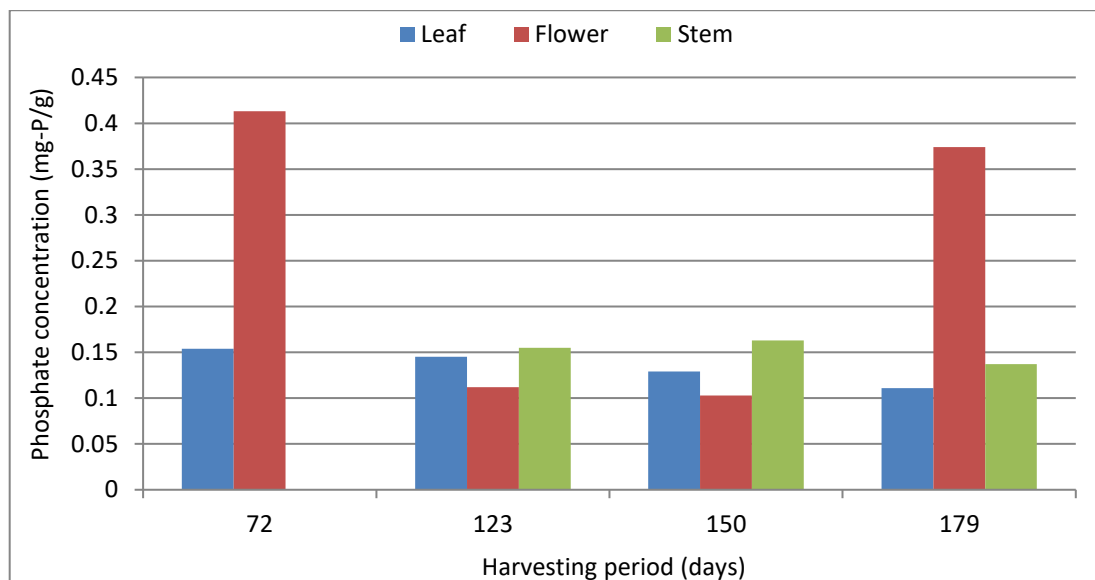


Fig. 12 Concentration of phosphate (mg-P/g) in plant tissues of *Canna indica* during each senescent phase.

Concentration of phosphate in tissue of dead leave and flower from first to fourth harvesting was observed as 0.154, 0.145, 0.129 and 0.111 mg-P/g, and 0.413, 0.112, 0.103 and 0.374 mg-P/g, respectively. Decrease in the phosphate concentration was observed with increase in the biomass of plant tissue. Dead stem was not present during first growth cycle. Concentration of phosphate in dead tissue of stem was observed as 0.155, 0.163 and 137 mg-P/g, from second to fourth harvesting, respectively. It was observed that first growth cycle was longest hence nutrient accumulation in leave and flower was found maximum. During growth period nutrient translocation is generally from bottom to top i.e. from roots to flower but in fall (autumn and winter) reverse translocation occur i.e. from flower to root (Verhoeven and Meuleman 1999).

#### Phosphate in sediments of *Canna indica* based cell

Results of phosphate concentration in sediments of *C. indica* based cells shows significant change during initial and final stage of experiment. Initial and final concentration of apatite, non-apatite and, total phosphate was observed to be varied from 64 to 138 mg-P/kg, 41 to 50 mg-P/kg, and 117 to 200 mg-P/kg, respectively, as shown in figure 13, which indicate that binding of phosphate with Fe/Al was higher than with Ca in present study.

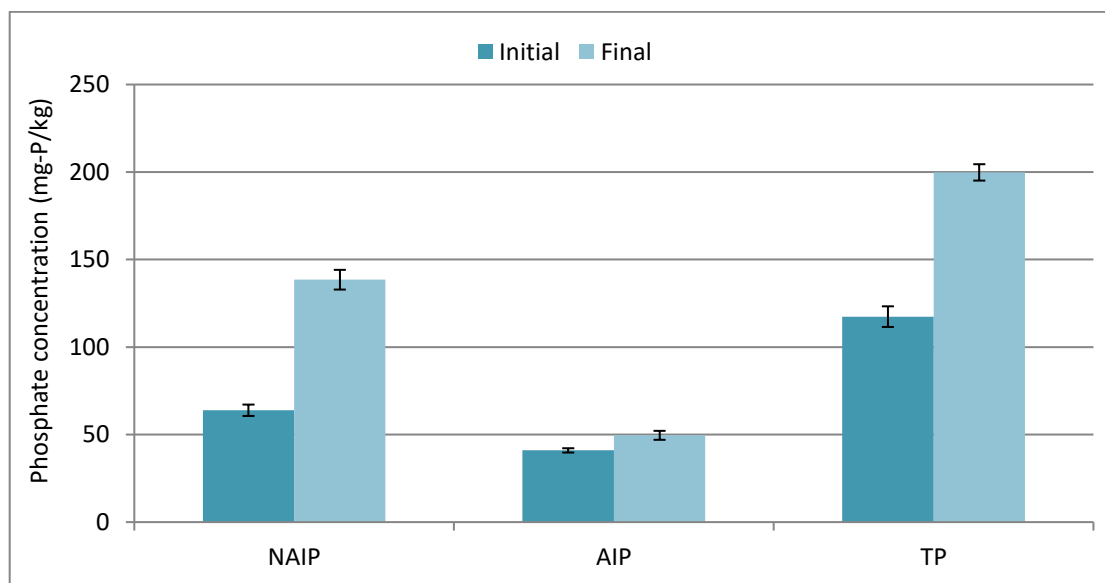
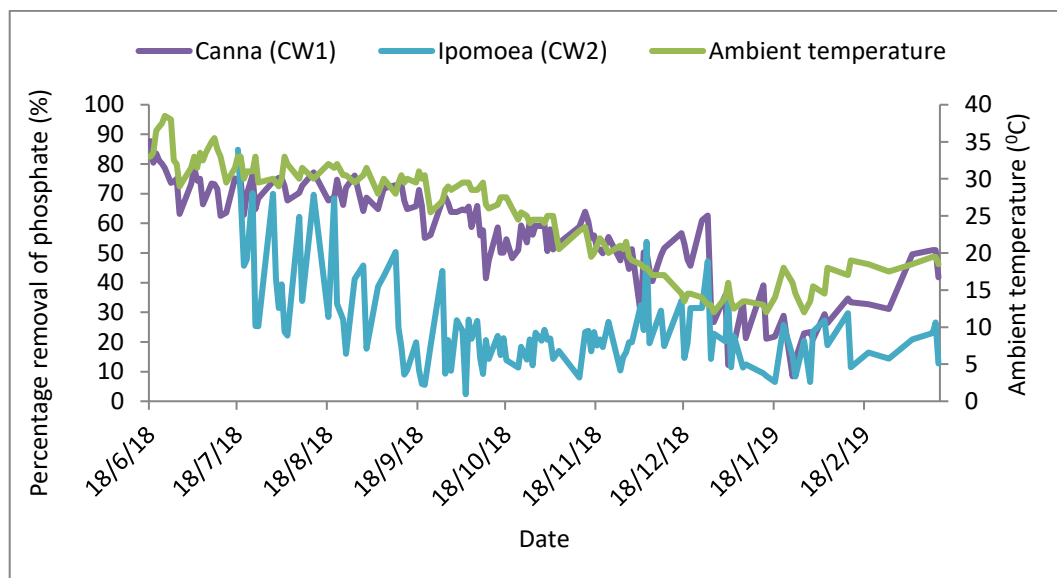


Fig. 13 Average concentration of apatite (AIP), non-apatite (NAIP) and total phosphorus (mg-P/g) in sediments of *Canna indica* based wetland (CW1) during initial and final phase of experiment.

Precipitation of phosphate with iron and aluminium ions leads to fixation of phosphates with metals, these processes have a much slower rate but are not so easily subject to saturation. If Ca adsorbed P is precipitated, the adsorption sites become available again for adsorption of new P (Verhoeven and Meuleman 1999). As pH of pore water in cell was below 8.0 (table 1) no significant change in the concentration of apatite phosphate was observed. pH on or below 7.0 has no or little impact on Fe/Al bound phosphate, but under limiting oxygen condition it can release from the sediments. In the present study redox potential was never fall below 0 mV, which can attributed to no release of Fe/Al bound P from sediments.

### **Influence of ambient temperature on phosphate removal**

Ambient temperature has direct impact on the evapotranspiration (ET) of the plant, with change in the temperature water uptake by the plants also changes. In summer or in hot weather as the ambient temperature is high plant ET is also high, thereby higher amount of nutrient is transfer with water in the plants. This transpiration reduces with lower ambient temperature hence the nutrient removal efficiency of plants also reduces. Variation of the removal efficiency of phosphate with change in ambient temperature has been shown in figure 14.



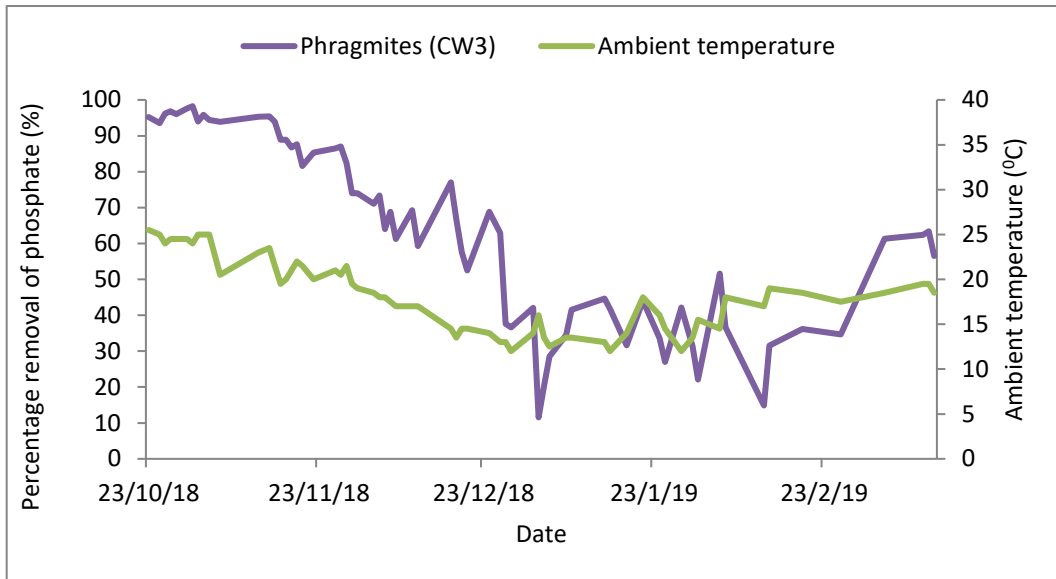


Fig. 14 Variation of percentage removal of phosphorus in different wetlands CW1 (*C.indica*), CW2 (*I. indica*) and CW3 (*P. australis*) with ambient temperature.

For all three plants percentage removal of phosphate was first reduced with ambient temperature and found minimum during winter (December and January) after that increase in efficiency was observed with increasing temperature during February and March. *Ipomoea indica* was found to be more sensitive plant due to higher fluctuation in removal efficiency. For both, *Canna indica* and *Pragmites australis*, percentage removal of phosphate varied directly with ambient temperature change.



## CONCLUSION

1. All three constructed wetlands were found suitable for the treatment of low strength waste water, highest efficiency was obtained during initial phase and reduction in efficiency was observed with time. Efficiency of wetland is affected by plant evapotranspiration (ET) which depends on meteorological factors like temperature, wind, humidity etc. Summer was recorded as the most favourable seasonal for constructed wetlands as the ET is higher in summer.
2. Broader leaves and soft tissue of *C. indica* allows more water to pass from it, that leads to more ET from *C. indica* than *I. indica* so water uptake with nutrients is more in *C. ndica*.
3. Leaf density of *P. australis* was higher than *C. indica* and its roots can penetrate deeper in the soil media hence water uptake with nutrients in *P. australis* is more. Hence, *P. australis* was found more suitable for phosphate removal than *C. indica* and *I. indica*.
4. As the nutrient loading in water bodies increases during summer (due to release from sediments, soil erosion through runoff off etc.), it requires treatment technology which has high efficiency during summer season and CWs are suitable for that.
5. Chemical composition of sediments affects the binding of phosphate in sediments. It was observed that fraction of Fe/Al bound P has increased in the sediments of CW1 due to the positive redox potential and almost neutral pH. This phosphate may release from sediments during adverse conditions.
6. Significant accumulation of phosphate was observed in the tissue of *C. indica*, *I. indica* and *P. australis*. It was observed that autochthonous addition of phosphate can be restricted through regular harvesting of dead tissue of plants.

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