Phytoremediation of Phosphates by Two Aquatic Macrophytes as a Remedy for Eutrophication

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Abstract:
Eutrophication, a globally concerned water quality impairment as a result of excessive nutrient discharge, primarily by phosphates to water bodies from agricultural and other anthropogenic origins. Beyond a threshold of >0.03 mgL^{-1} of phosphates, usage of such eutrophied water bodies leads to severe health and environmental concerns to adjacent communities and ecosystems. Phytoremediation is a cost-effective plant-based approach, has been identified as sustainable and environmentally friendly remediation. The broad objective of the study was to assess the efficacy of aquatic macrophytes for phosphate phytoremediation. *Eichhornia crassipes* (Water hyacinth) and *Pistia stratiotes* L. (Water lettuce) were selected as candidate macrophytes. The efficacy of two selected macrophytes was tested in ambient atmospheric conditions in a greenhouse using floating sieves. Phytoremediation efficacy of different contact times, introductory weights, pH values, and initial phosphate concentrations were assessed. The phosphate sequestration ability of *E. crassipes* and *P. stratiotes* were estimated. A fresh weight of 250±5 g of two macrophytes was introduced into to a 3 L of 25 mgL^{-1} of phosphate solutions. The phosphate removal efficiencies were 71.6% and 76.8% from *P. stratiotes*, and *E. crassipes* respectively, after 48 hrs of equilibrium time. The most effective introduction biomass was 550 g for both *P. stratiotes* and *E. crassipes* with removal efficiencies of 77.1% and 80.1%, respectively. Maximum removal efficiencies of 77.7% and 83.7% were observed for *P. stratiotes* and *E. crassipes* at pH of 7. *P. stratiotes* reached to its maximum removal efficiency of 88.2% in 25 mgL^{-1}, while in *E. crassipes*, the highest uptake was 47 mgL^{-1} at 250 mgL^{-1}, despite the highest removal efficiency of 89.5% was at 25 mgL^{-1}. *P. stratiotes* and *E. crassipes* showed a phosphorus sequestration potential of 35.4% and 41.6% from an eutrophied water body after five days, indicating a higher efficacy in phytoremediation and a candidacy of being a good source of phosphorus fertiliser in future.

Keywords:
Eichhornia Crassipes, Pistia Stratiotes, Phosphates, Phytoremediation, Removal Efficiency, Sequestration

1. Introduction

One of a most challenging environmental problem, the planet currently experiencing is eutrophication of water bodies. Despite the mechanism of eutrophication is unclear, the excessive nutrient accumulation in to surface water bodies have identified as a major factor triggering eutrophication [1]. A recent publication of World Health Organization defines eutrophication as a “Complex process which occurs both in fresh and marine waters, where excessive development of certain types of algae disturbs the aquatic ecosystems and becomes a threat for animal and human health” [2]. The primary cause of eutrophication is an excessive concentration of plant nutrients originating from agriculture or sewage treatments.

Over the past 50 years, the water dissolved nutrient levels of many water bodies (i.e. lakes and rivers) have augmented substantially [3]. During the last decade, this process was accelerated with amplified discharge of domestic wastes aided with rapid urbanization and non-point pollution from agricultural practices due to extraordinary increase of synthetic agricultural inputs [3]. Unarguably, eutrophication has become one of a most widespread environmental problem of inland waters. Unnatural enrichment of water bodies with basically two major plant nutrients, i.e. Phosphorus and Nitrogen disturb the aquatic biota by misbalancing growth, abundance and diversity. Amplification of these nutrients augments the natural balance of aquatic biota due to the rapid growth of aquatic plants and algae, clogging waterways and sometimes creating blooms of toxic blue green algae [4].

Eutrophication is substantial with increasing input rates of nutrient in a water body, due to rapid urbanization, industrialization and intensified agricultural production. Eutrophication in most fresh aquatic bodies is driven by phosphorus [4]. Agriculture, thus mineral fertilizers is considered as the main source of phosphorus, which accounts more than 95% of P accumulated in a water body [5]. Phosphorus and nitrogen, being key elements of eutrophication, current non-sustainable agricultural approaches require alternatives urgently for crop production [4]. Countering the growing food demands with exponential population growth, high input agriculture has become the savoir by boosting crop production in different dimension [6].

Excessive use of fertilizers, animal manures, or municipal wastes in forms of composts are primary reasons of phosphorus accumulation in soils. Accumulated P can travel via surface run off and sub-surface leaching towards water bodies in lower sections of the landscape. Hence, continuous phosphorus fertilization has become debatable with agronomic and environmental importance in various agricultural production systems [7].

During accumulation of P in a rapid rate, at first, the short living macrophytes grow much quicker and become larger comparatively to a non-eutrophied water body. Certain instances, phytoplanktons are also multiply. Rapid growth of macrophytes, such algae, and phytoplankton are low in density, thus float. Floating biota prevents a large proportion of the light from reaching to the lower layers of the bottom of the water body. Consequently, the first signs of reduction of oxygen concentration in deep layers become visible. With increasing severity, oxygen concentrations can reduce thus results aquatic life impossible. Subsequently, species which are capable of thriving in very little oxygen survive and further the conditions become detrimental for rest of aquatic biota in deep layers. Death biota increases the amount of organic sediments, leads to more anaerobic reaction; hence the final step is the end of all aerobic life.

Unnatural enrichment of nutrients also causes many deleterious impacts on human health, besides its effect on biodiversity and the ecosystem health. Certain algae and cyanobacteria species grow on eutrophied water bodies have the capacity of producing substances that are toxic to human beings. These toxins are found either free in the water, where the bloom occurs or bound to the algal or cyanobacterial cell [2]. In board sense, in all aspects, environmentally, ecologically, and socio-economically, the removal of phosphates from eutrophied water bodies is a must. Removal of P from water bodies can be done in many ways [8]. The conventional methods are like electrolysis, crystallization, filtration, chemical precipitation, biological treatments [8], reverse osmosis [9], coagulation [10], ion exchange [11], magnetic separation and constructed wetlands [12] have being widely tested for their efficacy and mass scale usability. Many have been failed due to poor operation stability or high economic cost at large scale [13].

Phytoremediation has been recognized as an economically viable chemical pollutants from contaminated soil, sediments or water. Phytoremediation is a cost effective and environmentally friendly process. Pollutants are removed by technology using green from contaminated sources by uptaking and then recycling. In general, phytoremediation yields better than conventional remediation methods. Phytoremediation has increasing scientific and commercial interest due to more environmental compatibility and cost effectiveness [14].

The primary objective of this study was to remove the phosphate from aqueous solution and develop a system applicable on large scale for removal of phosphate using phytoremediation. For this purpose, two aquatic macrophytes Eichhornia crassipes (Water hyacinth) and Pistia stratioes (Water lettuce) were used as phyto-accumulators for evaluation.

2. Materials and Methods

2.1. Establishment of Phosphate Removal Platforms

The basins were filled with 5 L of water and the desired volume of phosphate solution was added stirred well.

2.2. Instrumentation

The residual phosphate concentration was determined by a UV Spectrophotometer (Analytic Jena and Specord 210 plus, Germany). Ascorbic acid blue method [15] was used to prepare the ascorbic acid solution for colourimetiric phosphate analysis using an UV Spectrophotometer. Solutions were stirred on a hotplate stirrer (SB 162-3). Materials were weighted on an analytical balance and pH was measured by using a pH meter (HACH).

2.3. Phosphate Stock Solution Preparation

About 7 g of KH_2PO_4 (BDH, India) was oven dried at 105 ºC for 2 hours due to its hygroscopic nature. After allowing to cool inside a desiccator, 4.39 g was dissolved in distilled water and volume up to 1000 mL to prepare 1000 mgL^{-1} phosphate stock solution. Desired concentrations of phosphate solutions ranging from 5 mgL^{-1} to 250 mgL^{-1} were prepared by diluting the stock solution.

2.4. Ascorbic Acid Reagent Preparation and Colorimetric Determination of Phosphorus

Defined quantities [15] of Ammonium molybdate, 2.4M Sulphuric acid, Ascorbic acid and Antimony potassium tartrate were measured using pre-cleaned and 1% acid treated measuring cylinder. Precisely prepared 1 mL of ascorbic reagent was added to 12 mL of water sample that was prepared for analysis of phosphorus and allowed for 30 mins for the color development. The color intensity was measured at 880 nm wavelength using UV Spectrophotometer (Analytic Jena and Specord 210 plus, Germany). The respective concentration was obtained using pre-prepared standard curve.

2.5 Preparation of plants

Eichhornia crassipes (Water hyacinth) and *Pistia stratioes* (Water lettuce) plants were collected from surrounding naturalized communities, where these plants are abundant. Surface dirt, algae, and aged root tissues were removed from the plants. Cleaned plants were transferred and grown in open tanks under prevalent conditions. Plants were selected for uniform size, weighed and introduced to water containers with 11 L of water added with 0.5 M Hoagland nutrient solution. After introduction of aquatic plants, experiments were maintained in a greenhouse in ambient temperatures (28-30 ºC).

2.6. Determination of Optimal Operational Parameters

2.6.1. Effect of Contact Time

Weighed, pre-cleaned plants were introduced into containers with 3 L of water supplemented with 25 mgL^{-1} phosphate solution. The solution pH was maintained at 5.5-6.5. Water samples for residual phosphate analysis was obtained in time intervals of 3, 6, 9, 12, 24, 48, and 54 hours, after introduction. The time taken to establish an equilibrium for absorption was determined. The experiment was replicated three times. Two control experiments were prepared with phosphate solutions i.e. without aquatic plants and i.e. without added phosphate in the same experimental conditions. Phosphorus was determined a using the colourimetric method [15].

2.6.2. Effect of pH

Weighed pre-cleaned plants were introduced into containers with 3 L of 25 mgL^{-1} phosphate solution. The pH levels of the solutions were adjusted to 4, 5, 6, 7, 8, and 9 with 3M HCl and 3M NaOH solutions. At the time of the equilibrium, samples were obtained for the residual phosphate analysis. Experiment was replicated three times. Two control experiments were prepared with phosphate solutions *i.e*. without aquatic plants and *i.e.* without added phosphate in the same experimental conditions for corrections. Phosphorus was determined a using the colourimetric method [15].

2.6.3. Effect of Initial Plant Mass

Pre-cleaned plant masses of 50, 150, 200, and 500 g were grown in separate containers with 3 L of 25 mgL^{-1} phosphate solution. At the time of equilibrium, samples were obtained for residual phosphate analysis. The experiment was replicated three times. Two control experiments were prepared with phosphate solution i.e. without aquatic plants and only plants i.e. without added phosphate in the same experimental conditions for corrections. Phosphorus was determined a using the colourimetric method [15].

2.6.4. Effect of Initial Phosphate Concentration

A range of phosphate concentrations from 10 to 250 mgL^{-1} was prepared in containers. Pre-cleaned and weighed plants were introduced in to 5 L of above concentrations, separately. At the time of equilibrium, samples were obtained for residual phosphate analysis. The experiment was replicated three times. Two control experiments were prepared with phosphate solution i.e. without aquatic plants and only plants *i.e.* without added phosphate in the same experimental conditions for corrections. Phosphorus was determined a using the colourimetric method [15].

2.7. Determination Phosphate Sequestration by Aquatic Plants

A system was re-established with optimum optimal operation parameters. Initial phosphate of plants was determined before the introduction. Pre-cleaned plant masses were introduced to the established systems and removed at the equilibrium time.

The final phosphate level in plant tissues was determined. Plant tissue phosphorus was determined by the method described in [16]. A digestion mixture was prepared by adding 0.21 g of Selenium powder, 7 g of Lithium sulphate, 175 mL of 30% Hydrogen Peroxide and 210 mL of concentrated Sulphuric acid. Digestion mixture of 4.4 mL of digestion mixture was added to 75 mL digestion tubes with 0.2 g of dried and ground plant materials. Samples were digested at 360 ºC for 2 hrs and allowed to cool. About 50 mL of distilled water was added to the tubes and mixed well. The solution was volumetrically made up to 100 mL. A clear solution of 12 mL was obtained, and phosphorus was determined a using the colourimetric method [15].

2.8. Calculating the Phosphate Absorption Efficiency and Sequestration

The absorption of phosphates from the solution was determined with the following equation.

$$\text{Phosphate uptake efficiency} = C_i - C_f / C_i * 100 \quad (1)$$

Where; Ci = Initial phosphate concentration

Cf = Final phosphate concentration [17]

The phosphate sequestration was calculated using the following equation.

$$\text{Phosphate Sequestration} = FP - I\,P / Wp * 100 \quad (2)$$

Where; FP= Final phosphate in plant ($g\ kg^{-1}$)

I P = Initial phosphate plant ($g\ kg^{-1}$)

Wp= Initial phosphate in solution ($g\ kg^{-1}$)

2.9. Statistical Analysis

Means and standard deviations were calculated for all parameters. Differences of phosphate removal were determined by analysis of variance and Tukey's HSD test at $p < 0.05$.

3. Results

3.1. Effect of Contact Time

Initially, the phosphate uptake by both macrophytes was substantially low; however, the uptake tended to increase with time, especially after around 1000 minutes (~16 hrs) (Figure 1). The uptake had reached to an equilibrium level, where there was no increasing trend after 48 hrs. The maximum phosphate uptake equilibrium for both macrophytes was approximated at 48 hrs (Figure 1).

Pistia stratiotes was comparatively low in efficiency in phosphate uptake than *Eichhornia crassipes* although the initially the rates were more or less equal. Prior to the equilibrium (at 48 hrs), the uptake rates were significantly high in *E. crassipes*, while the difference of uptake was narrowed towards the equilibrium. In *P. stratiotes*, the uptake had reached to the maximum uptake of ~4.18 mgL^{-1}, while in *E. crassipes* the uptake was 4.46 mgL^{-1}. The mean uptake values were similar ($p=0.14$) for both macrophytes at 48 hrs equilibrium stage.

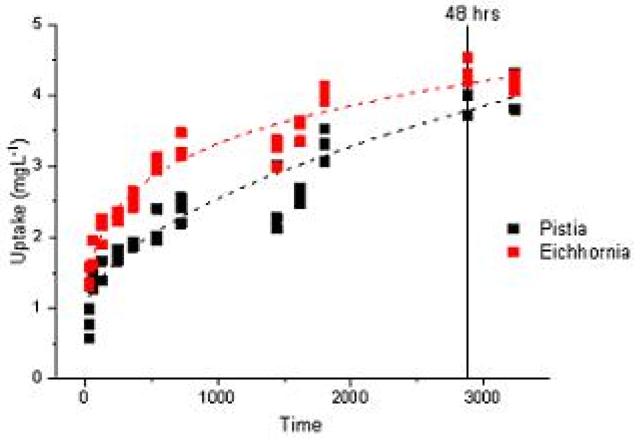

Figure 1. *Phosphate uptake by two aquatic macrophytes in different time intervals.*

3.2. Effect of pH on Phosphate Uptake

The two macrophytes performed differently in corresponding pH levels. However, for both species, the highest phosphate uptake was observed at pH 7. The uptake efficiencies were 83.7% and 77.7% for *Eichhornia crassipes* and *Pistia stratiotes* respectively, as a fraction of initial solution phosphate concentration in pH 7 (Table 1). Hence, for these two macrophytes pH 7 was identified as the optimum pH for phosphate uptake.

Generally, towards acidic pH, the uptake was significantly low compared to the highest, in both species, despite the quantitative similarity of uptake of both at low pH. Similar drop of phosphate uptake was observed with increasing alkalinity; however, the reduction was slow in *Pistia stratiotes* when compared to *Eichhornia crassipes* (Table 1).

At pH 4, *Eichhornia crassipes* showed its lowest phosphate uptake with an efficiency of 55.3% compared to the efficiency at neutral pH. At pH 8, the uptake efficiency was around 61.1% compared to the efficiency at neutral pH. A reduction of 22.6% in uptake efficiency was evident, when the pH of the medium changed from neutral to slightly alkaline. Hence, for *Eichhornia crassipes* the optimum operational pH was in-between 6.5 to 7.5.

Table 1. *Mean phosphate uptake by two macrophytes from acidic to alkaline ranges of pH after introducing 250 g of fresh biomass of each species to 3 L of phosphate solution with 25 mgL^{-1} concentration.*

Plant species	Mean uptake at any pH (mgL^{-1})	Mean pH	Mean Plant uptake (mgL^{-1})
Eichhornia crassipes CV %	3.82 3.95	4	3.14 ^{c}
		5	3.27 ^{c}
		6	4.46 ^{a}
		7	4.76 ^{a}
		8	3.47 ^{b}
Pistia stratiotes CV %	3.94 3.95	4	3.29 ^{c}
		5	3.53 ^{b}
		6	4.17 ^{b}
		7	4.46 ^{a}
		8	4.24 ^{a}

Means followed by the same letters are not significantly different at $p<0.05$

Pistia stratiotes showed a similar uptake compared to *E. crassipes* in slightly acidic medium (pH 4 & 5) (Table 1). However, the uptake efficiencies were the lowest (57.4% and 61.5%, respectively) compared to the highest observed at neutral pH. The highest uptake of *P. stratiotes* was observed in neutral medium, which was similar to in *E. crassipes* (Table 1) with an uptake efficiency of 77.7%. Contrastingly, at peak uptake, *P. stratiotes* showed 15% more uptake compared to *E. crassipes*.

P. stratiotes showed a higher ability in uptaking phosphate from a slightly acidic and an alkaline media than *E. crassipes*. *P. stratiotes* showed slightly higher efficiency of 0.8% in slightly alkaline medium and a higher efficiency of 2.1-3.9% in slightly acidic medium. Hence, for *Pistia stratiotes*, the optimum operational pH was in-between 6.5 to 8.5.

3.3. Effect of Introductory Biomass on Phosphate Uptake

The phosphate uptake was increased with the introductory plant biomass for both species. The lowest mean uptake was recorded at 50 g of biomass (Table 2) with uptake efficiencies of 31.05% and 38.93% by *P. stratiotes* and *E. crassipes*, respectively.

E. crassipes showed a distinct grouping among biomasses of 50, 150 and 200 g, where the uptake was lower than 1.0 mgL^{-1} from the highest (Table 2). The highest uptake was observed from 550 g introductory fresh weight; however, no significant difference was observed between the introductory fresh weights of 450 g. The uptake efficiencies were 78.86% and 80.13%, respectively for 450 g and 550 g for introductory biomasses.

The rate of phosphate uptakes from biomass levels of 50 g to 450 g had been increased at an increasing rate (i.e. from 0.56 mgL^{-1} g^{-1} to 1.14 mgL^{-1}g^{-1}), and then the rate was slowed (0.07 mgL^{-1}g^{-1}) from 450 g to 550 g for *E. crassipe*. However, *P. stratiotes* resulted comparatively low mean phosphate uptake. From 200 g of introductory biomass to 450 g, the uptake had changed significantly by more than 1.4 mgL^{-1}, where the same difference between the same biomasses of *E. crassipes* was much lower.

The phosphate uptake, at low fresh weights and nearly up to 250 g was rapid but comparatively slower afterwards in *E. crassipes*. Around fresh weight of 450 g, the uptake had become constant. In *P. stratiotes,* a rapid uptake was observed up to 400 g fresh biomass and then after the uptake was slower, while at 500 g of biomass, the uptake remained constant. The uptake by *E. crassipes* was always higher than *P. stratiotes* for introductory biomass (Table 2).

Table 2. *Mean phosphate uptake by two macrophytes with changing introductory fresh biomass of each species to 3L of phosphate solution with 25mgL^{-1} concentration.*

Plant species	Mean plant uptake at any biomass (mgL^{-1})	Biomass (g)	Mean Plant uptake (mgL^{-1})
Eichhornia crassipes CV %	3.55 4.18	50	2.26 ^{d}
		150	2.82 ^{c}
		200	3.44 ^{b}
		450	4.58 ^{a}
		550	4.65 ^{a}
Pistia stratiotes CV %	3.21 6.36	50	1.80 ^{c}
		150	2.44 ^{b}
		200	2.92 ^{b}
		450	4.39 ^{a}
		550	4.48 ^{a}

Means followed by the same letters are not significantly different at p<0.05

3.4. Effect of Concentration on Phosphate Uptake

The highest solution phosphate concentration resulted the highest plant uptake of *E. crassipes*. The uptake was significantly higher from the rest (Table 3). Despite the higher plant uptake at 250 ppm, the uptake efficiency was low (18.8%) with respective of the initial concentration of the solution. In contrary, the lowest uptake was observed at concentration of 10 ppm, while it had yielded an uptake efficiency of 82.59%. The highest uptake efficiency (89.5%) was observed at 25 ppm of initial phosphate concentration. At high phosphate concentrations, both macrophytes showed low uptake efficiencies.

Table 3. *Mean phosphate uptake by two macrophytes after introducing 250 g fresh biomass of solutions of different phosphate concentrations to 3L of phosphate solution.*

Plant species	Mean uptake at any concentration (mgL^{-1})	Concentration (ppm)	Mean Plant uptake (mgL^{-1})
Eichhornia crassipes CV%	22.44 27.55	10	8.43 ^{c}
		25	22.50 ^{b}
		50	14.44 ^{bc}
		100	19.85 ^{bc}
		250	46.99 ^{a}
Pistia stratiotes CV%	20.76 36.44	10	8.09 ^{b}
		25	22.98 ^{a}
		50	19.95 ^{ab}
		100	27.35 ^{a}
		250	25.44 ^{a}

Means followed by the same letters are not significantly different at p<0.05

In *P. stratiotes*, the lowest uptake was observed at 10 ppm, yet the uptake efficiency remained at its highest with 80.36%. Similar to *E. crassipes*, the highest efficiency recorded was 88.23% at 25ppm. Uptake at 25, 100 and 250 ppm were statistically similar (Table 3), nevertheless the respective uptake efficiencies were varied in contrast. The uptake efficiencies were 25.9% and 10.4%, at 100 and 250 ppm, which were substantially compared to the uptake efficiency of 25 ppm.

Generally, *Pistia stratiotes* and *Eichhornia crassipes* were similar in low phosphate concentrations, while *E. crassipes* was superior in high phosphate concentrations.

3.5. Phosphorus Sequestration of the Two Macrophytes

Eichhornia crassipes showed the highest phosphorus sequestration out of the two macrophytes (Table 4). The sequestration ability of both macrophytes had reduced with increasing number of days in the solution. After 5 days, the sequestration and sequestration efficiency had reached to a peak for both species. Interestingly, in *E. crassipes*, a significant reduction in sequestration was observed after 5th day in the phosphate solution till the 10th day. Meantime, the sequestration efficiency was also reduced nearly by 18%, when compared to the sequestration at 5th day. Then, the sequestration had increased till 15th day after introduction, but at a slower phase compared to the 1st five days (Table 4). The lowest sequestration level and the sequestration efficiency were observed in plants harvested after 10 days of exposure to phosphate solution. Cumulative sequestration and cumulative sequestration percentages of two macrophytes have increased up to 10 days and reduction around ~0.5 in cumulative sequestration can be observed.

Table 4. *Mean phosphate sequestration, cumulative phosphate sequestration and sequestration efficiency by two macrophytes after introducing 150 g fresh biomass of each species in to 3L of phosphate solution with 25 mgL^{-1} concentration and with pH of 7.*

Plant species	***Eichhornia crassipes***			***Pistia stratiotes***		
Mean Phosphorus Sequestration at any day (g/day)	2.6			1.7		
Days	5	10	15	5	10	15
Mean Phosphate uptake (mgL^{-1})	22.16 ^{a}	16.06 ^{b}	12.00 ^{b}	19.49 ^{a}	13.41 ^{b}	12.44 ^{b}
Mean Phosphorus sequestration † (g/day)	3.4 ^{a}	1.9 ^{b}	2.4 ^{ab}	2.9 ^{a}	0.9 ^{b}	1.3 ^{b}
Sequestration %	41.6	23.5	29.3	35.4	10.9	16.2
Cumulative Phosphorus sequestration (g/day)	3.4	4.9	4.4	2.9	4.9	4.5
Cumulative Sequestration %	41.6	59.8	54.0	35.4	59.9	54.5

Means followed by the same letters are not significantly different at $p<0.05$
*†Phosphorus was calculated from Phosphate using (Molecular weight of P/ Molecular weight of PO_4^{-3}) * respective PO_4^{-3} concentration*

In *Pistia stratiotes*, the highest sequestration was observed at 5th day, whereas the lowest was observed at 10th day after introducing to the solution. A significant drop in phosphorus sequestration was observed from 5th to 10th day, while there was a gain afterwards, but it was low and not significant. In contrast, *P. stratiotes* was less efficient than *E. crassipes*, however the highest sequestration efficiency was observed in 5th days after introduction (Table 4). The sequestration efficiency was reduced more than 24% in 10thday, which was higher than the reduction observed from *E. crassipes.*

4. Discussion

Phytoremediation, which uses of plants to remove contaminants from environment, has gain increasing attention in many ecological studies [18]. Hence, many researchers focus on diverse studies based on phytoremediation. Many studies had done for evaluating the potential efficiency of different plant species for the remediation purpose [19] and applicability of different phytoremediation systems at field level, which was also the fundamental concept of this study.

4.1. Superiority of Aquatic Macrophytes

Two aquatic macrophytes were chosen based on the literature [19], [20] assuming their phosphate uptake or phytoremediation capabilities can be fitted to the dynamic conditions of different aquatic ecosystems. The potential of these two macrophytes in remediation has been proven by many studies. *E. crassipes* was used in remediating textile effluent and found that 52.9% phosphate reduction [21]. While [22], has observed 40-55% ortho phosphorus reduction from wastewater by *E. crassipes*. Meantime, [23] has reported 81.6% reduction of phosphorus from contaminated water within 10 days by *P. stratiotes*. *E. crassipes* and *P. stratiotes* were identified as the most and widely used aquatic macrophytes in water contaminant removal [24], [25) [26]. Despite having many advantages in phytoremediating (i.e. cost effective, environmentally friendly, low maintenance), the technology is limited due to time-consuming nature of it with the long-life cycle of most plant species used for phytoremediation [27]. *E. crassipes* and *P. stratiotes* show a rapid growth and landscape spread, as these are non-indigenous invasive plants, thus the biomasses are doubling in a very rapid rate [28]. The potential applications of non-indigenous invasive, free floating aquatic macrophytes like *E. crassipes* and *P. stratiotes* for nutrient removal from contaminated water have been widely reported by [29] and [30]. *E. crassipes* were utilized in nutrient rich wastewater and were impressive in removal rates of inorganic nitrogen [nitrate (NO_3^- N), ammonium (NH_4^+ N), and total N)] and phosphorus (PO_4^{-3} P and total P). The wide range of application of these two species are due to the rapid, vigorous growth and high biomass yield. Simultaneously, the ability of the two plants in hyperactive accumulating N and P in tropics and sub-tropic environment [31].

4.2. Effect of pH on Phosphate Uptake

The level of phosphorus removal and its efficiency are mainly depending on the growth and development of a plant, where in an aquatic ecosystem, the quality of water is the key. The growth of these aquatic plants is highly sensitive to the temperature and pH of the growing media [32], hence these two parameters significantly control the bio-removal of nutrients. In addition, the solubility, availability and uptake of phosphorus are also depending highly on pH level of the system compare to other nutrients responsible for plant growth [33].

The best pH range for the growth of *E. crassipes* is 5.5-7.0 [34] and at neutral pH the plant has its' optimal growth, but it can tolerate pH values from 4 to 10 [35]. The pH was changed from 4 to 8 during the experiment, and the highest phosphate uptake was observed at pH 7 (Table 1). As stated, [36], the plant has increasing nutrient uptake at neutral and slightly alkaline pH (pH 7 and pH 8), which probably the optimum for biomass production. *P. stratiotes* has the ability of converting alkaline pH to neutral [37] by absorbing of nutrients and other salts [38]. No any significant difference was observed in P uptake in pH level 7 and 8, by *P. stratiotes* indicating the similarity to the previous studies. The pH is playing a significant role in determining uptake as the growth always assisted especially with generation of new roots for maintaining an accelerated uptake. In certain aquatic systems, where the pH is in either one of extreme, the selection of plant species is always critical [39]. For neutral pH, these two macrophytes were the ideal as it was demonstrated.

4.3. Introductory Plant Masses and Uptake

Phosphate uptake of the two macrophytes was also varied with the introductory biomass and the initial ion concentration of the solution. High plant biomass of both macrophytes, the uptake had been increased (Table 2). High biomass always linked to greater area of root surface for effective absorption and high leaf area for greater transpiration [40]. Plant biomasses have a direct impact on the phytoremediation process by affecting directly the plant root surface binding capacity and its uptake capacity. The larger amount of biomass provides more surface area for the phytoremediation processes [40], thus with higher efficiency. Even in an aquatic ecosystem, with much more extreme conditions, high introductory biomass is beneficial as the survivals of such plants are guaranteed with more energy that an individual plant carry.

4.4. Effect of Solution Phosphate Concentration on Uptake

The uptake efficiency of the two macrophytes had increased to 80.36% to 88.23% and 82.59% to 89.46% in P. stratiotes and *E. crassipes* respectively with initial concentration of 10 and 25 ppm. However, the uptake efficiency decreased when the phosphate concentration was higher as 50 ppm. Similar results were obtained by [41], for nitrates. Extremely high PO4-3 and increasing it further may affect in an antagonistic manner, a thus the nutrient uptake diminishes with high osmotic pressure of the solution [42]. A repulsion of the anions by negatively charged cell wall [40] leads to low uptake, thus low efficiency. As stated by [43], at lower concentrations of heavy metals, the plant growth was normal and removal efficiency was greater, which may also be true for phosphorus as phosphorus career sites are the mechanism of heavy metal uptake too. The differences in uptake with increasing concentration among the two macrophytes clearly indicate that the absorption capability and tolerance to high phosphate concentration by *E. crassipes* was comparatively higher than P. stratiotes. With increasing concentration from 0 ppm to 40 ppm, *E. crassipes* has absorbed greater amount of Phosphorus and that Phosphorus has become uniformly distributed throughout the plant [39]. Increase nutrient uptake without consequent increase in growth has been reported to *E. crassipes*, but the upper limit of phosphate absorption is unknown [44]. The larger total root surface, higher root activity, active absorption area, root biomass, leaf area and net photosynthetic rate of *E. crassipes* is much greater than in P. stratiotes has resulted comparatively high removal efficiency [45].

4.5. Phosphorus Sequestration

A higher sequestration efficiency of both macrophytes was observed five days after plant introduction. When the retention time increases up to 10 days, the efficiency was decreased, however by retaining another 5 days (total of 15 days), the efficiency was marginally increased (Table 4). *P. stratiotes* plant may tend to accumulate large quantity of nutrients initially during their rapid growth and rapid multiplication [46]. After 15 days, the absorbed nutrients start to release to the water body with the decaying of basal leaves [46]. Hence, periodic harvesting of *P. stratiotes* is essential to remove nutrients effectively for phosphorus and also for n nitrogen too. Similarly, heavy metals that absorbed from contaminated water can release again to water with root and leaf decaying process [47,48].

E. crassipes has a very efficient nutrient removal nutrient mechanism, when plants are young and in rapid growing phase, thus regular harvesting is necessary. Exceeding the appropriate time of harvesting, absorbed nutrients can release back to water due to necrosis and decaying of older plants and parts [41]. *E. crassipes* may remove around 60 kg of Phosphorus if plants are harvested during maximum growth, from 0.40-hectare area [49]. The present study showed that around 10 mg of phosphate per 1 kg of dry weight fixed in *E. crassipes*, while, it was 8 mg of PO_4^{-3} per 1 kg of dry weight within 5 days in *P. stratiotes.* These sequestrations were from an initial phosphate concentration of 25 $mgkg^{-1}$.

Biomass of *E. crassipes* is rich with essential nutrients like nitrogen and phosphorus, hence this aquatic macrophyte is a good candidate as a fertiliser after decomposition. In addition, after harvesting, *E. crassipes* can be used to produce methane via anaerobic digestion, as green manure, ash for regenerating degrade soils and as fodder for livestock, because it is rich in proteins [50]. At present, the commonly used green manure Alfalfa (*Medicago sativa* L.) is the highest P containing green manure with 0.3-0.42% of its dry weight, where in other plants it is about 0.05-0.3% [51]. In the present study, *E. crassipes* yielded 0.31% and *P. stratiotes* yielded 0.26% (based on dry weight) of Phosphorus, five days after introduction. Hence, two macrophytes showed a great avenue in fulfilling nutrient requirement, especially phosphorus for crop growth, if used as mixture of two species, where in future, phosphorus from depositions may hard to come by.

4.6. Phytoremediation by Eichhornia Crassipes and Pistia Stratiotes

Both *E. crassipes* and *P. stratiotes* were highly efficient in removing P (Table 4), which is in-lined with the efficient N and P removal by *P. stratiotes* from eutrophic storm water in constructed water detention shown by [52]. *E. crassipes* can attain the highest nutrient removal efficiency from synthetic solutions [41], when compared with other test plants. Both free floating aquatic macrophytes can serve as good phytoremediators, but when compared to *P. stratiotes*, *E. crassipes* showed rather higher phosphate uptake. However, in a study done by [18], had obtained vice-versa, where results showed *P. stratiotes* had a removal capability of 58.27% of total phosphorus compared with *E. crassipes* when phytoremediating of contaminated water with domestic sewage. The nature of phosphorus in water may determine the differential absorption, adsorption and precipitation, in addition to the pH of the water body as it can be varied with the major source of pollution [53]. The dense and longer roots in aquatic macrophytes are beneficial in accumulating, intercepting and precipitating higher amount of phosphorus [54]. The use of plant roots to intercept contaminants has been identified as one of the major process of phytoremediation [55].

In contrary, most these polluted or eutrophied water bodies are also rich in anthropogenic heavy metals. Especially, water bodies near urban areas show a tendency in accumulating more Lead, Arsenic and Cadmium like heavy metals [19]. This has been a greater bottleneck of phytoremediating urban water bodies with aquatic marcophytes and reusing them as composted materials. Very similar to the phosphate/ phosphorus uptake, most of these heavy metals are also absorbed via same mechanism or same carriers of phosphorus. Hence, there is always a risk of contaminating composted aquatic macrophytes, despite being a good phosphorus source for crops. Pre-caution is essential, and also proper quantification is also essential for heavy metals, in such situation; disposing of harvested macrophytes should be very responsible.

Based on the present study, it has been observed that both macrophytes have a great avenue in remediating eutrophied water. Previous studies suggested that use of only one floating aquatic macrophyte at once could not provide desired management of contaminated aqua systems [56]. A combination of two macrophytes will provide better results hence for that further studies including these two species is essential [18].

5. Conclusions

Eichhornia crassipes and *Pistia stratiotes* are good candidates for phytoremidiating eutrophied aquatic water bodies with strict management conditions may also substitute other expensive physicochemical methods for phosphate removal. Introducing both in would be ideal but by having a better understating of the condition, especially pH and phosphate concentration, one of these macrophyte can be chosen. The sequestration is substantial, and comparable with other phosphorus sources by green manure. There is a potential of using harvested macrophytes as a rich source of phosphorus for fertilising cropping fields after decomposing. In future, where mined phosphorus sources will be scare, which urge recycling of phosphorus responsible for eutrophication. Hence, in future eutrophied water bodies are very good sources of phosphorus and remediating these water bodies will be a must for the sake of environment and for agriculture. However, the approach shall be cautious as both of these macrophytes are super absorbents of heavy metals. Further, both of these macrophytes are categorized as non-indigenous invasive plants, hence a utilizing them for an economically viable process may also handy in controlling their landscape spread.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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