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The Efficiency of Aquatic Macrophytes on the Nitrogen and Phosphorous Uptake from Pond Effluents in Different Seasons

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ABSTRACT

The present study investigated the efficiency of four aquatic macrophytes: *Lemna spp, Pistia stratiotes, Ipomoea aquatica* and *Eichhornia crassipes* on nitrogen and phosphorous utilization from aquacultural effluents concerning seasonal changes and biomass production. These nutrients in excess affect fish health and cause eutrophication in water bodies, hence affecting the ecosystem. Aquatic macrophytes were planted in tanks filled with the effluents from carp pond and other tanks were left without plants, serving as control/algal treatment. The water samples were collected weekly for analysis of total nitrogen (TN), ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), total phosphorus (TP) and ortho-phosphate (ortho-P). The results show that average water temperature raised from 12.2 ± 0.21 °C in winter to 32.0 ± 0.4 °C in summer with no significant difference ($p>0.05$) between treatments whereas pH was neutral in winter and slightly alkaline in the other seasons. Seasonal changes had impact on macrophytes biomass accumulation with the highest in spring for *Lemna spp* (91.3%), followed by *P. stratiotes* (81%) and in summer, *E. crassipes* (64%). Autumn and winter had the lowest biomass accumulation and *I. aquatica* had the lowest values in all seasons. For each season, the nutrients concentration decreased with no significant difference ($p>0.05$) between treatments. Average NH_3-N removal efficiencies were higher during summer and autumn followed by spring and lowest in winter for all treatments. NO_3-N and TN decreased significantly from the highest in summer to the lowest in winter in all treatments. The ortho-P removal efficiency was slightly higher than TP and decreased from the highest in spring to the lowest in winter (91.4% to 7.8%, control/algae; 90.3% to 8.4%, *E. crassipes*; 86.2% to 8.3%, *Lemna spp*; 82.5% to 10.8%, *P. stratiotes*). The chlorophyll a concentration was higher in *Lemna spp* (62.2 μg/L) and control/microalgae treatments (59.3 μg/L) indicating that there was probably microbial community that contributed to nutrient utilization. Aquatic macrophytes, in association with microalgae, were responsible for the nitrogen and phosphorous removal. Seasonal temperature change affects the growth and nutrients uptake of aquatic macrophytes. A decrease in temperature reduces the efficiency of nutrients removal and biomass production. For an effective N and P removal from pond effluents in a given season, selection of a proper aquatic macrophyte must be taken into consideration with regards to a given season.

Keywords: biomass, nutrients, phytoremediation, pond effluents, seasonal variations.

INTRODUCTION

Aquatic macrophytes are widely used around the world in constructed wetlands for the purpose of removing N and P from polluted water Vymazal, [2013]. Several studies were conducted regarding the use of aquatic macrophytes on treating wastewater from different sources; for example, Lu, Fu, & Yin, [2008]; Reddy & DeBusk, [1985] used *E. crassipes* to treat effluent from duck farm, Tabinda et al., [2019] used *P. stratiotes, E. crassipes* and *Oedogonium sp.* on textile effluents, *I. aquatica* was used on quality improvement for aquaculture wastewater Zhang, Achal, Xu, & Xiang, [2014]. In addition, duckweed (*Lemna* spp) also showed good results in wastewater treatment, Al-Qutob & Nashashibi, [2012]; Liu, Dai, & Sun, [2017]; Toyama, Hanaoka, Tanaka, Morikawa, & Mori, [2018].

Before applying the aquatic plants for wastewater treatment, it is important to understand the characteristics of these plants on their effectiveness on wastewater treatment. The ecological issues are another thing to be considered in a targeted site, since this process might affect the ecological relationships of other plants, Mahmood, Mirza, & Shaheen, [2015]. An aquatic plant or aquatic macrophyte can be either emergent, submergent or floating that grows and obtains its nutrients in or near water and sometimes can be found in a marsh like helophytes, or partly submerged in water, Beentje, Hickey, & King, [2001]. Floating macrophytes are not dependent on soil or water depth while submerged or merged ones depend on both. They tend to cover the water surface and block out the passage of light to the water below, denying algae to grow and reproduce by limiting the energy supply. In most lakes or rivers that are polluted due to nutrients loading, aquatic macrophytes grow naturally and use these nutrients for their growth and form large biomass, which in return can be used for economical purposes, Reddy & De Busk, [1985]. Many aquatic macrophytes and other terrestrial plants were found to be hyper-accumulators or accumulators of organic as well as inorganic contaminants in different polluted areas Jatav and Singh, [2015].

One of the well-known and important functions performed by macrophytes is the uptake of dissolved nutrients such as N, P, heavy metals etc. from highly polluted water and are widely used in constructed wetlands around the world to remove excess nutrients and heavy metals Chen, Wen, Zhou, & Vymazal, [2014]; Dhir, Sharmila, & Saradhi, [2009]; Iamchaturapatr, Yi, & Rhee, [2007]; Nandakumar, Pipil, Ray, & Haritash, [2019]; Oladejo, [2018]; Zhang, Sun, Xie, Wu, & Cheng, [2018]. In comparison with terrestrial plants, aquatic plants are known to have faster growth, larger biomass production, relatively higher capability of pollutant uptake and better purification effects Fernández, Fernández-Pascual, Mañero, & García, [2015]; hence, they are good candidates for removal of nutrients. Using aquatic plants for wastewater purification is cost-effective due to their availability and ability to survive under adverse conditions with their robust formation of colonies, Aisien, E.T., Aisien, F.A. and Gabriel, [2015]; Sa'at & Zaman, [2017]. Though freshwater aquatic plants have high capability of removing nitrates and phosphorous from water, their response to this depends on the species used during treatment, Sooknah & Wilkie, [2004]. Chen et al., [2018], reported that various plant species perform differently in terms of the nitrogen and phosphorous uptake at various period i.e. different weather. These plants can be useful in aquacultural industry in different ways: they can be used to treat pond effluent as in the presented study, can be used as feed for fish directly or after processed into fish feed and also can provide breeding environment for fish and other aquatic organisms associated with fish. In some countries, some of these macrophytes are used as food for human beings, e.g. water spinach.

Four aquatic macrophytes; *E. crassipes, Lemna* spp*, P. stratiotes* and *I. aquatica* were selected to analyse their effectiveness in removing N and P from the fishpond effluent and biomass production in different seasons in the present study. The obtained results will a clear aspect on which macrophytes can be used in a given season for pond effluent treatment. The conducted study aimed to 1) evaluate the effects of seasonal change on the N and P uptake rates by aquatic macrophytes; 2) assess the role of seasonal change on macrophytes Biomass production; and 3) determine which individual macrophyte has the best N and P removal efficiency in a given season. In the case of nutrients removal, aquatic macrophytes perform differently with regard to the weather conditions, [Chen et al., 2018]; hence it is good to understand which plants can be used in a given season.

MATERIALS AND METHODS

Experimental facilities and wastewater source

A total of 15 recirculating tank with carrying capacity of 1200L of water and surface area of 2 m^2 were used. Prior to water fill, the tanks were cleaned and left to dry, then they were filled with wastewater from fish ponds up to approximate volume of 700L and allowed to recirculate within the tanks for two days. After recirculating, all the inlet and outlet taps were closed in each tank allowing water to settle ready for aquatic plants stocking and for each season fresh wastewater was filled into the tanks.

Aquatic Plants

A total of four aquatic plants, namely: water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna* spp), water spinach (*Ipomoea aquatica*) and water lettuce (*Pistia stratiotes*), were used as phytoremediators in this study. Water hyacinth, duckweed and water lettuce were obtained in the wastewater pond found inside the school while water spinach was purchased from the nearby market and raised till the roots appeared. The aquatic macrophytes were collected, then washed with fresh water prior to be stocked in a divided pond supplied with freshwater for acclimatization and removal of any nutrients in the plant within one week. After acclimatization they were weighed and transferred to their corresponding tanks.

Experimental Setting

The experiment took place in April 2019 (Spring), July 2019 (Summer), October 2019 (Autumn) and January 2020 (Winter), completing four seasons of the year. A complete randomized design was adopted in which five treatments with three replicates (1, 2, 3) were used in each treatment. The treatments were assigned as: Control treatment (CT: 1, 2, 3), *Eichhornia crassipes* treatment (EC: 1, 2, 3), *Pistia stratiotes* treatment (PS: 1, 2, 3), *Lemna* spp treatment (LS: 1, 2, 3) and *Ipomoea aquatica* treatment (IA: 1, 2, 3). On the basis of the growth rate and surface area coverage, the average wet biomasses of the selected aquatic plants were 300.2 g, 300.8 g, 50.2 g and 251.0 g for *E. crassipes, P. stratiotes, Lemna* spp. and *I. aquatica,* respectively.

Physical Characteristics of Wastewater

The water quality measurements were done after collection of water samples starting from day 0 in the interval of 4 days in each experimental period. Water temperature and water pH were determined by using Mettler Toledo™ SevenExcellence™ S400 pH/mV Meter (USA) and were measured within one hour after collection. Dissolved oxygen (DO) was measured directly in experimental tanks by using DO meter (HACH HQ30d Portable meter flexi, USA).

Sample collection

Water samples for nutrients analysis

The water samples for nutrients determination were collected once per week; in each tank water was collected from three different points and mixed together to make a 1L sample. Capped plastic containers were used for water sample collection after been washed with distilled water and were labelled according to the treatments. The collected samples immediately were taken to the laboratory for analysis; if not so, the samples were stored in the refrigerator at 4 °C, all analyses were performed within 48 hrs.

Plants Biomass

Plants biomass were measured at the beginning and end of each experimental time using electronic balance scale. Plants were removed from water, then placed in quadrat net covered with a filter paper to remove excess water for about five minutes, the remaining water was dried by using a tissue paper, then weighed and their wet weights were recorded. The initial and final biomasses obtained were used to determine their growth rate, relative growth rate (RGR) and biomass accumulation. The RGR was calculated using the following equation:

$$RGR = \frac{lnW_2 - lnW_1}{t_2 - t_1} \tag{1}$$

where: W_2 and W_1 are final and initial weights, respectively, and t_2 and t_1 are time.

Other plants samples were analyzed for TN as total Kjedahl Nitrogen (TKN) and TP in their roots and leaves for *P. stratiotes, E. crassipes* and *I aquatica* while *Lemna* spp., the whole plant was analyzed.

Chemical analysis

Chlorophyll 'a' analysis

Monitoring the chlorophyll levels is the direct method of tracing algal growth, since it is known to be essential in the existence of phytoplankton or algal present in surface water, Higgins, [2014]. Chlorophyll 'a' was determined using the spectrophotometric method, where-by the water sample was filtered using filter membrane. Then, the membrane was placed in a centrifuge tube and stored in the refrigerator at 4 °C for at least 6h and extracted using 90% acetone (v/v) solution and centrifugation at 8000 rpm for 8 min. Supernatant was poured in a cuvette then measured at wavelengths (630, 647, 664, and 750 nm), calculations were performed based on the equations provided by Jeffrey & Humphrey, [1975]. The following formula was used to calculate the final concentration on chlorophyll concentration:

$$\rho = [11.85 * (A664 - A750) - 1.54 * (A647 - A750) - 0.08 * (A630 - A750)] * V1/(V * \delta) \quad (2)$$

where: ρ - mass concentration of chlorophyll ‘a’ in water sample (μ/L), V1 - Constant volume of extract (ml), V - volume of water sample (L) and δ - Cuvette path (cm)

Nutrients analysis

The chemical analyses of total ammonia nitrogen (TAN), total nitrogen (TN), nitrate-nitrogen (NO_3-N), ortho-phosphates (ortho-P) and total phosphorous (TP) were performed using the standard methods, APHA, [2017]. Specifically, TAN was determined by using the Salicylate method (HACH method 10023) with detection range of 0.02 to 2.5 mg/l NH_3-N at wavelength of 655 nm. NO_3-N was determined by means of the Chromotropic Acid method (HACH method 10020) with detection range 0.2 to 30.0 mg/L NO_3-N at 500 nm and USEPA PhosVer® 3 with Acid Persulfate Digestion method (HACH method 8190/ Standard Methods 4500-P E) was used for TP determination with detection range of 0.06 to 3.5 mg/l PO43- at 880 nm wavelength. All the HACH methods were detected using DR2800 spectrophotometer HACH, Germany. TN was determined by using the persulfate digestion method, Ortho-P by means of the Ascorbic acid method (EPA 365.2+3/APHA 4500-P E) using Spectroquant® prove test kit, Merck Millipore, USA. The obtained data were used to calculate the nutrient removal efficiency with the following formula:

$$Removal\ efficiency\ (\%) = \left(\frac{C_0 - C_1}{C_0}\right) * 100 \quad (3)$$

where: C_0 and C_1 are initial and final concentration respectively.

Data analysis

All data including, water parameters, nutrients concentration, plant biomass were entered into MS Excel spreadsheet [2016] for calculations. Analysis of variance (ANOVA) was conducted by using SPSS 25.0 statistical software to compare means between the treatments, and LSD at $p<0.05$ was considered significant [IBM Corp., 2017]. All figures were made by using OriginLab 2018 package.

RESULTS AND DISCUSSION

Water quality Characteristics

Throughout the present study, the pH values were almost the same in all seasons with no significant difference and ranges from 8.5 ± 0.07 to 9.1 ± 0.26 in spring, 8.0 ± 0.0 to 9.0 ± 0.2 in summer, 8.3 ± 0.06 to 8.5 ± 0.05 in autumn and in winter was little lower than the other seasons 7.5 ± 0.10 to 8.1 ± 0.14. The conducted observations on constant pH within seasons were similar with those obtained by [Xu et al., 2019; Yang, Yan, Wang, Zhang, & Wang, 2019]. Therefore, the pH values did not affect the performance of the aquatic macrophytes. Unlike pH, water temperature and DO were fluctuating within the seasons with high temperatures observed in summer and the lowest was observed in winter and DO was high during summer, see Figure 1. The presented results were not far from those obtained by [Yang et al., 2019], in which the amount of dissolved oxygen and temperature in water were varying within seasons, while pH was not affected by seasonal changes. The optimum temperature for *Lemna* spp. is between 17.5 °C to 30 °C [Leng, 1999] while for *P. stratiotes* it ranges from 22 °C to 30.3 °C, and their growth stops at 8 °C–15 °C [Sooknah & Wilkie, 2004]; in turn, the optimal temperature for *E. crassipes* is between 25.0 °C and 27.5 °C [Gray, 2004]. Therefore, according to the obtained results, the average temperature for spring favors the growth of *Lemna* spp., *P. stratiotes,* and a slightly *E. crassipes*. The summer temperature was good for *E. crassipes* and autumn was favorable for *P. stratiotes*. The temperature ranges between 25 °C to 35 °C are suggested to be suitable for microbial processes such as nitrification during wastewater treatment pure cultures, [Akinbile & Yusoff, 2012] while Shah et al. in their study, when using water hyacinth, water lettuce and duckweed, suggested that temperature between 15 °C and 38 °C is suitable for their performance, [Shah, Hashmi, Ghumman, & Zeeshan, 2015]. In addition, total phosphorus and total nitrogen removal in floating wetlands occurred when the air temperature was between 5 °C to 15 °C and the removal drop when the temperature was higher or lower, [Van De Moortel, Meers, De Pauw, & Tack, 2010]. Concerning those previous studies, the water parameters observed in the performed research were suitable for macrophytes’ performance.

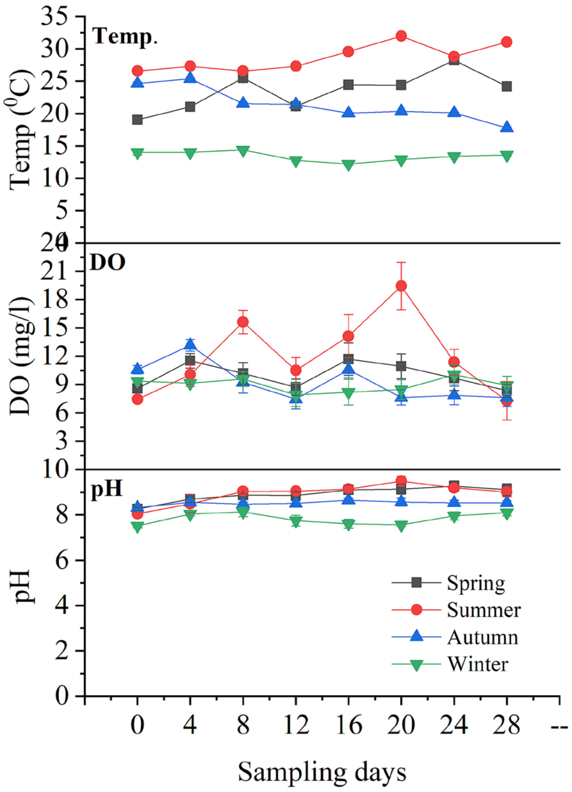

Figure 1. Water parameters; Water temperature (°C), pH and Dissolve oxygen (mg/l) observed in different seasons. Results are presented in mean ± SD which is represented as error bars (n=3)

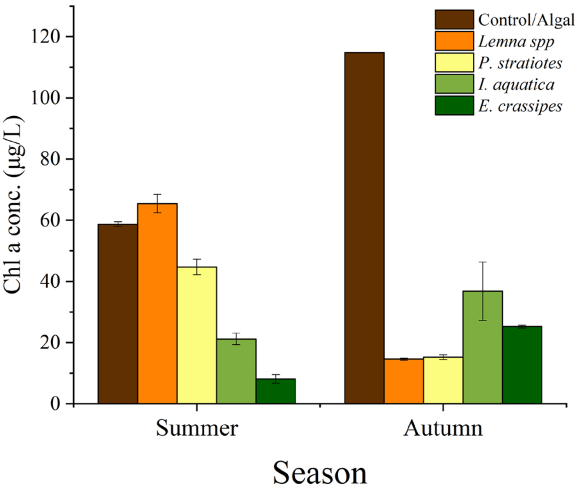

Figure 2. The concentration of chlorophyll a, in pond effluents during summer and autumn seasons (n=3)

Chlorophyll-a concentration

A high content of Chl-a was observed in the control/algae and *Lemna spp* treatment followed by *P. stratiotes* in summer (Fig. 2). The concentrations were: 115.8 µg/L (Control/algae), 14.5 µg/L (*Lemna* spp), 14.9 µg/L (*P. stratiotes*), 22.6 µg/L (*I. aquatica*) and 23.3 µg/L *for E. crassipes*. The highest chlorophyll-a concentration observed in control/algae treatment proves the presence of microalgae and phytoplankton were responsible for nutrients reduction. This suggestion was previously reported by Li et al., [2011] in their study, where nutrients removal from wastewater was due to the algal growth.

Biomass production

There was a positive effect of nutrients toward plant biomass; as the nutrients decreased in water, the plant biomass was increasing, meaning aquatic macrophytes were utilizing the nutrients in water (Table 1). The biomass accumulation obtained showed high variation between seasons and among the macrophytes (Figs. 3, 4). The accumulation trend during spring was 91.3%, 81.0%, 58.2% and 17.5% for *Lemna* spp*, P. stratiotes, E. crassipes* and *I. aquatica,* respectively. During summer, biomass accumulation for *E. crassipes* (64.1%) and *I. aquatica* (23.2%) increased significantly as compared to the previous season, while *P. stratiotes* and *Lemna* spp dropped. Autumn and winter experienced lower biomass accumulation than the other two seasons, with winter having the lowest accumulation in macrophytes. *I. aquatica* has negative accumulation in autumn and could not survive in winter. Overall, the results for *P. stratiotes* and *Lemna* spp were good in spring and they covered the whole take area, while in summer it was *E. crassipes* followed by *P. stratiotes* and *I. aquatica* could not cross 50% of removal efficiency in all seasons. The biomass production of the four aquatic macrophytes differed within seasons, indicating that season variations with reference to temperature change play part in growth performance, [Yang et al., 2019]. The observation from the present study showed that the productivity of *Lemna* spp and *P. stratiotes* were favored by the temperature during spring time, while that of *I. aquatica* and *E. crassipes* were limited, since they could not stand the low temperature. During summer, *Lemna* spp could not survive the high temperatures, showing reduced growth followed by death after the second week when the temperature ranges from 26 °C to 32 °C and that of surrounding were around 39 °C. On the other hand, *I. aquatica* and *E. crassipes* have increased growth rate, as compared to the spring season while *P. stratiotes* was decreasing significantly and some of the plants were dying.

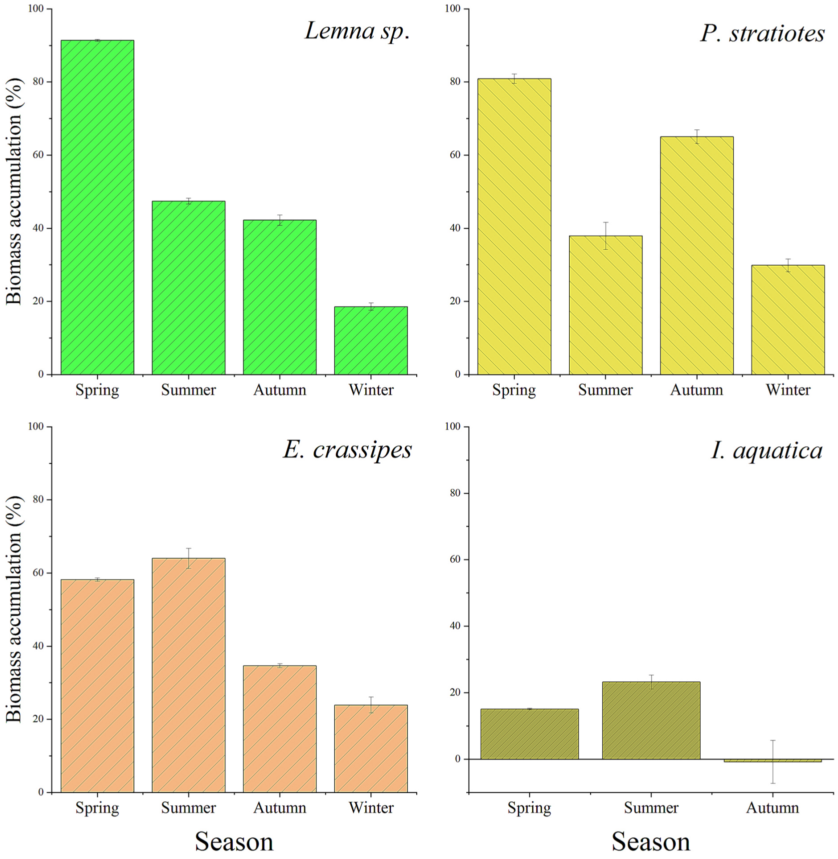

Figure 3. Change in biomass accumulation (%) of the four aquatic macrophytes in each season

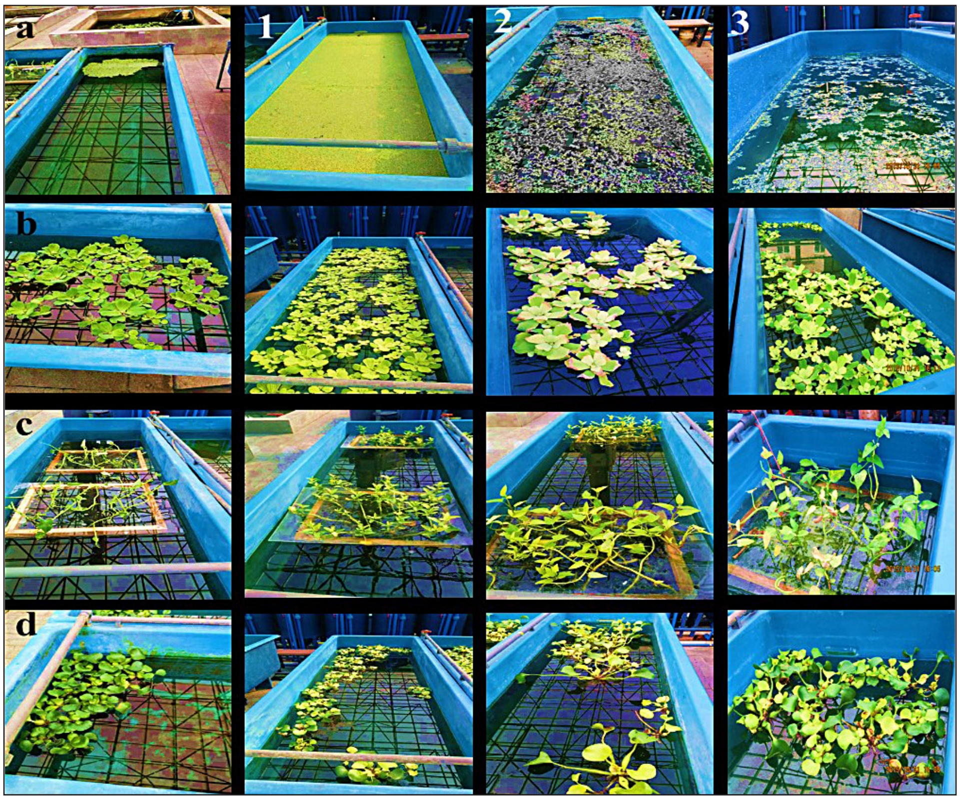

Figure 4. Aquatic macrophytes growth and coverage. Row; (a) *Lemna spp*, (b) *P. stratiotes*, (c) *I. aquatica* and (d) *E. crassipes*. Column; First column represents initial biomasses, 1-3 Final biomasses in spring (1), summer (2) and autumn (3)

Nutrients Removal

Nitrogen removal

The nitrogen removal is mainly done through plants uptake of associated microorganisms attached to their roots i.e. rhizosphere, volatilization of dissolved ammonia to the atmosphere and by chemical reactions; nitrification and denitrification, [Amare, Kebede, & Mulat, 2018; Marimon, Xuan, & Chang, 2013]. Nitrogen was analyzed in the form of ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N) and total nitrogen (TN) for weekly removal rate and seasonal removal efficiency. In the present study, the nitrogen removal concentration in water was decreasing as time goes on in all treatments with no significant difference (p>0.05) between the groups in all seasons Figure 5. The NH_3-N removal efficiencies during the spring season were 94.87%, 84.62%, 61.54%, 46.15% and 38.15% for *E. crassipes, P. stratiotes, I. aquatica, Lemna* spp and control/algal group, respectively. During summer and autumn all treatments have high NH_3-N removal efficiencies with the overall average being 97.5% in summer and 98.0% in autumn. Winter has the lowest NH_3-N removal, as compared to the other seasons in which *P. stratiotes* has the highest

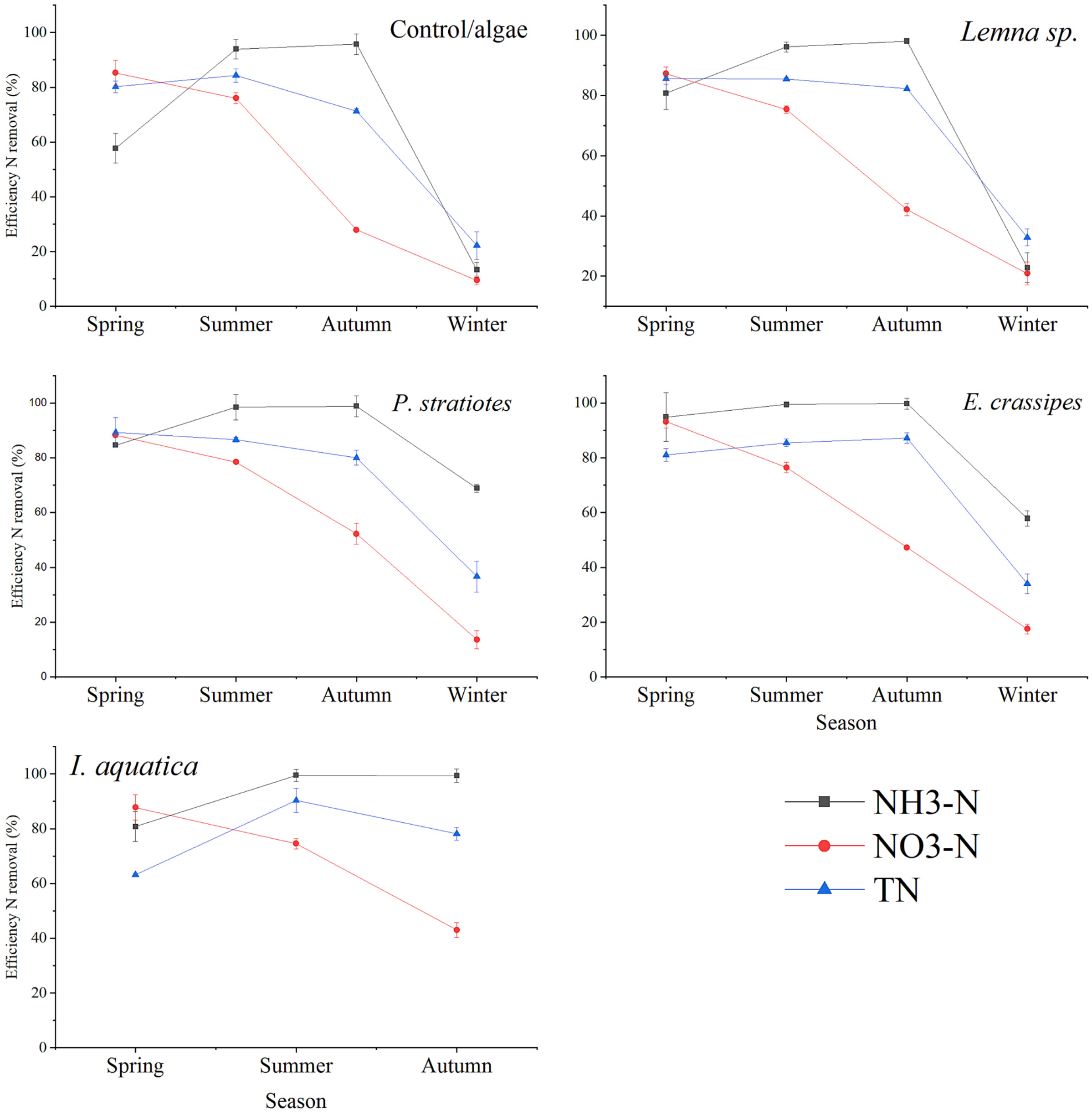

Figure 5. Nitrogen forms (NH_3-N, NO_3-N and TN) removal efficiencies (%) in different seasons. The relative standard deviations are indicated as error bars (n=3)

removal efficiency (63.89%) and the lowest was control/algal treatment (5.17%). The obtained results in summer and autumn were similar to those [Lu, Xu, Li, & Chai, 2018; Sooknah & Wilkie, 2004], in which they reported 96% and 99.6% for *E. crassipes* and 93% and 99.2% for *P. stratiotes* respectively, and their average temperature range were 20.4 °C to 30.3 °C and 28 °C to 36 °C. [Zhang et al., 2014] reported 60% ammonia removal by *I. aquatica* which was similar to this study.

Unlike NH_3-N, NO_3-N and TN decreased from the highest in summer to the lowest in winter (Fig. 5) with a significant difference between the treatments. This suggests that temperature change has affected the N removal from pond wastewater as it is an important parameter required by the aquatic plants in facilitating nutrients uptake. Temperature played a vital role on Total Kjedahl Nitrogen (TKN) during summer and spring on their study observations in which and the lowest TKN removal efficiency was observed in spring, [Nandakumar et al., 2019]. Higher temperatures around 38 °C temporary affect the nitrification process, [Sarioglu et al., 2017] and the optimal temperature for nitrifying bacteria growth is between 25-35 °C, [Hu, Yuan, Yang, & He, 2010]. Regarding the obtained results between spring to autumn, the high nitrogen removal efficiency was observed especially for TN and NO_3-N indicating that how seasonal changes concerning temperature affect the nutrients removal efficiency.

Phosphorous removal TP

The phosphorous removal efficiencies by aquatic macrophytes were decreasing seasonally, with the highest removal in spring to the lowest in winter, in both analyzed forms of phosphorous, see Figure 6. The removal efficiency during spring season for ortho-P was 91.4%, 90.3%, 86.2%, 82.5% and 80.5% for control/algae, *E. crassipes, Lemna* spp*, P. stratiotes* and *I. aquatica,* respectively, with no significant difference between treatments (p>0.05). From summer, the removal efficiencies decreased in all treatments, in which control /algae treatment dropped from 91.4% in spring to 83.7%, (summer), 56.8% (autumn) and 7.8% in winter, for *E. crassi*pes were 80.3% (summer), 52.4% (autumn) and 8.4% (winter). In turn, the removal efficiencies for *P. stratiotes* were 82.5% (summer), 52.7% (autumn) and 10.8% (winter) and 78.0% and 48.7% in summer and autumn respectively for *I. aquatica.* The TP removal efficiencies were a little lower than ortho-P in all treatments for each season and not significantly different. The highest and lowest removal observed for each season were, in spring: *E. crassipes* (85.9%), and *I. aquatica* (73.1%), in summer: control/algae (79.8%) and *E. crassipes* (66.4%), in autumn: control/algae (57.2%) and *I. aquatica* (27.1%) and in winter which was overall had the lowest removal efficiencies as compared to the other seasons, *E. crassipes* (17.0%) and control/algae (7.5%). The results obtained by [Sudiarto, Renggaman, & Choi, 2019], show that *E. crassipes* can remove 87.94% of TP from treated swine wastewater at a temperature range from 25 °C-27 °C. The TP removal by *I. aquatica* was 27.5%, [Zhang et al., 2014] which were similar to those obtained in this study during autumn and the main mechanism of removal was through assimilation. The high P removal observed in the control/algae treatment is due to the presence of microalgae which play a vital role in P reduction and were confirmed by the increase in Chl a concentration in water. In addition, macrophytes roots were found to have higher contents of P than leaves, which confirm the plant uptake (the results are not included). The same observations were also reported by [Di Luca, Mufarrege, Hadad, & Maine, 2019], the P concentrations were significantly higher in roots and rhizomes than in the aerial parts of *Typha domingensis***.** Furthermore, as stated by [Spieles & Mitsch, 1999], removal of total phosphorous is not affected by temperature, its removal is mainly influenced by sedimentation, adsorption and microbial activities.

CONCLUSIONS

Seasonal temperature change has an impact on the performance of aquatic macrophytes. According to the obtained results, all aquatic macrophytes were involved in nutrients removal from pond effluents through direct uptake and microbial processes. Among the four macrophytes, water lettuce (*P. stratiotes*) had large biomass accumulation in spring and autumn, so it can be used during these two seasons. Moreover, Duckweed (*Lemna* spp) is recommended to be used in spring for better biomass production and nutrients removal, while in summer water hyacinth (*E. crassipes*) is a good choice. None of the four macrophytes are recommended in winter, so it is

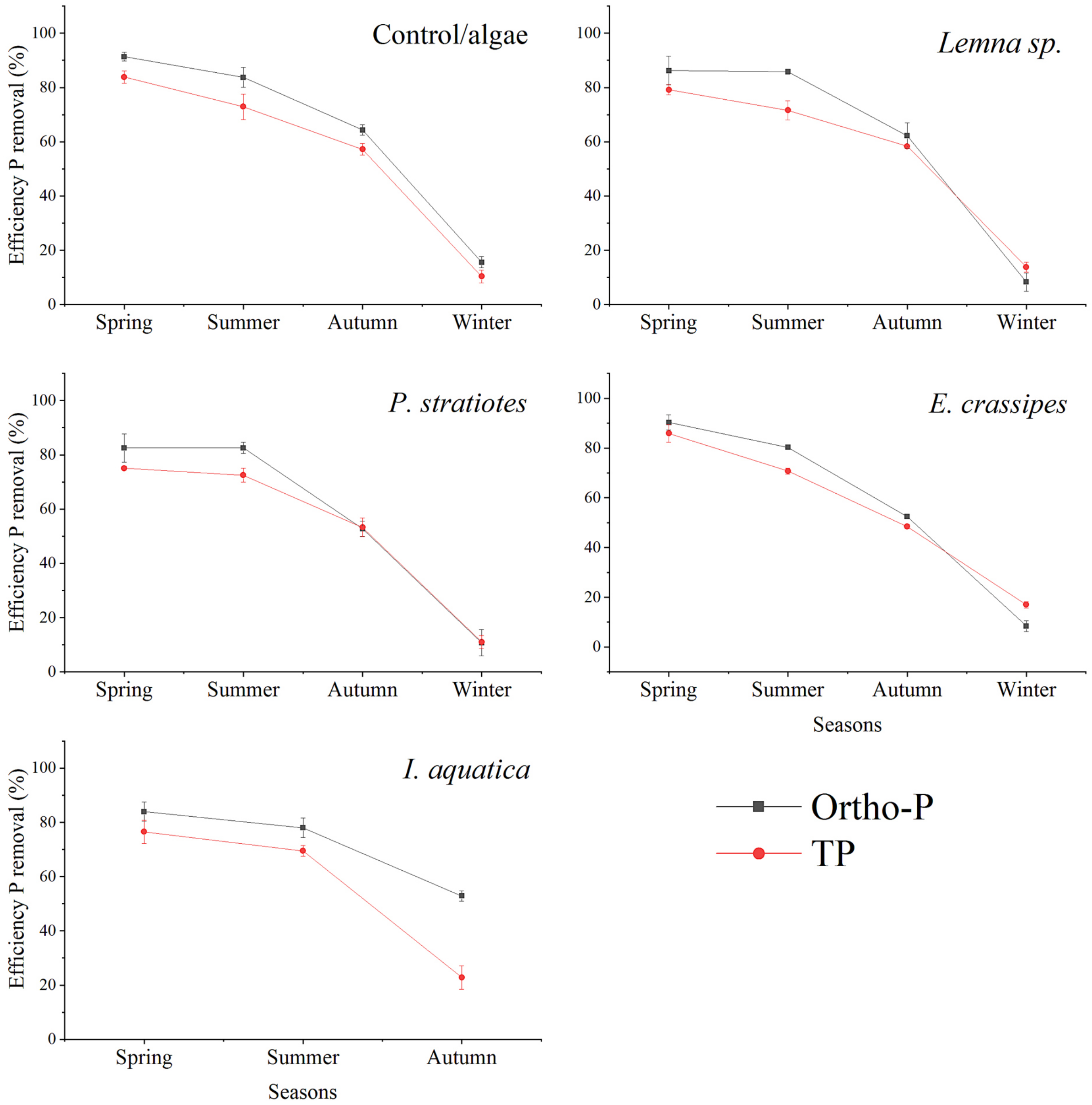

Figure 6. Ortho-phosphate and total phosphate removal efficiencies by aquatic macrophytes in different seasons. The relative standard deviations are indicated as error bars (n=3)

better to find another alternative plant that will tolerate cold weather. *I. aquatica* was not a good candidate in terms of biomass production in all seasons, but plays a role in nutrients removal through uptake, as their roots grew denser while shoots and leaves did not.

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