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Artificial Floating Islands for the Removal of Nutrients and Improvement of the Quality of Urban Wastewater

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Abstract: A high amount of nutrients can be found in urban wastewater (UW), which makes it difficult to treat. The purpose of this research was to evaluate the potential of the aquatic macrophytes *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia molesta* in the treatment of UW. To evaluate the potential of each macrophyte, phytoremediation bioassays were established; the hydraulic retention time for each bioassay was 15 days. The physicochemical analysis of the water samples considered pH, turbidity, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand (COD), total carbon (TC), phosphates (PO_4^{3}-P), nitrate (NO_3-N), and total nitrogen (TN). To evaluate the phytoremediation potential of each plant, the bioconcentration factors (BCFs) and translocation factors (TFs) for NO_3-N and PO_4^{3}-P were evaluated. Likewise, the relative growth rates (RGRs) and total chlorophyll production of the macrophytes were measured. The results showed that the highest efficiency was achieved with the bioassays with *E. crassipes*, with removal values of 69.7%, 68.8%, 58.7%, 69.4%, 56.3%, and 40.9% for turbidity, COD, TOC, PO_4^{3}-P, NO_3-N, and TN, respectively. The phytoremediation potential results showed that, for BCF, the highest value was 4.88 mg/g of PO_4^{3}-P with *E. crassipes*, and for TF, it was 6.17 mg/g of PO_4^{3}-P with *S. molesta*. The measurement of RGR and total chlorophyll for *E. crassipes* showed an increase of 0.00024 $gg^{-1}d^{-1}$ and an increase of 4.5%, respectively. On the other hand, the other macrophytes suffered decreases in chlorophyll content and RGR. Thus, *E. crassipes* is defined as the macrophyte with the greatest potential for the UW phytoremediation process.

Keywords: phytoremediation; floating macrophytes; eutrophication; nutrient pollution

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1. Introduction

In recent decades, the world's population has grown at an astonishing rate, and cities have been constantly growing, causing a serious shortage of fresh water [1,2]. According to the United Nations, around two-thirds of the world's population currently faces water scarcity [3]. On the other hand, as urbanization accelerates, wastewater-related problems increase due to the discharge of untreated wastewater into various water bodies [4]. Pollution reaching freshwater bodies in urban areas is a serious global problem [1].

Urban wastewater contains large amounts of nutrients (nitrogen- and phosphorus-based compounds); when these waters are discharged into bodies of water, their effect is harmful, directly affecting the deterioration of the trophic state of freshwater [2]. One of the harmful effects is eutrophication; this occurs when there is an excessive presence of nutrients, causing the proliferation of algae, some of which release toxic substances, such as cytotoxins, which represent a threat to humans, fish, and shellfish, as well as marine mammals and birds [5,6]. Eutrophication can also change the composition of species in an ecosystem, resulting in the loss of vital ecological services [7]. Additionally, the economic impacts of eutrophication are significant and can include increased water treatment costs and loss of recreational opportunities [7,8]. Mitigation of eutrophication in freshwater bodies has been tested with a wide range of technical and regulatory approaches [9–11]. One of the factors that most limits the use of these techniques is usually their high cost [12]. This is why, in the last 40 years, plant-based treatment methods such as phytoremediation have had a greater impact. Compared with biological and chemical treatment processes, phytoremediation processes are more feasible and effective in most cases [13–15]. Phytoremediation is a plant-based process that absorbs or degrades excess nutrients in terrestrial and aquatic environments. It is a profitable, sustainable, and environmentally friendly technology; it can be used in artificial wetlands or hydroponic systems [16]. Artificial floating islands (AFIs) are a variation of constructed wetlands [17]. They are structures designed to float on the surface of a body of water and structured to develop plants whose roots grow at the bottom of the water [18,19]; the vegetation can imitate natural wetlands and perform multiple physical, chemical, and biological functions. For this reason, they have been tested as an alternative for the treatment of wastewater from different polluting sources [20–22] and used as an ecotechnology to mitigate eutrophication and improve water quality [18]. AFIs have gained popularity as low-cost solutions [22].

AFIs are constructed using emergent and free-floating aquatic macrophytes, which provide valuable habitats and theoretically improve ecological function [23]. The hydroponic nature of floating macrophytes presents a significant advantage over traditional phytoremediation treatment systems (constructed wetlands), since, in their extensive floating rhizospheres, a greater surface area is available for biofilm growth [24]. Macrophytes that have been used in wastewater phytoremediation include the following: *Eichhornia crassipes*, *Pistia stratiotes*, *Ceratophyllum dermesum* L., *Potamogeton perfoliatus*, *Lemna minor*, *Limnobium laevigatum*, *Typa orientalis*, *Vertiveria zizaniodes*, *carrizo común*, *maná*, *malva de Virginia*, *Salvinia molesta*, *Stuckenia pectinate*, *Phragmites australis*, *Alternanthera*, *Arundo donax*, *Mentha Aquatica*, *Nelumbo nucifera*, and *Nymphaea* [16,25]. In particular, the macrophytes *E. crassipes*, *P. stratiotes*, and *S. molesta* have a significant advantage over other plants, due to their exceptional nutrient uptake, resistance to pollution, and massive growth. Considered hyperaccumulator plants, they are efficient in capturing chlorides, sulfates, nitrates, phosphates, carbonates, and heavy metals [26,27]. However, the mechanisms of absorption, translocation, and transformation in these plants are not yet fully characterized [28], such that there is disagreement about the nutrient elimination pathways (absorption or sedimentation). Recent review articles have attributed the majority (50.8%) of nutrient removal to sedimentation [29] and have concluded that "sedimentation caused by the root system is the primary route for removal" [20]. However, there is no consensus on the criteria for determining nutrient capture pathways. It is for all the above reasons that the objective of this study was to evaluate the performance of *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia molesta* regarding the removal of NO_3-N and $PO_4{}^3$-P from urban wastewater using artificial floating islands.

2. Materials and Methods

2.1. Description of the Study Area

The urban wastewater (UW) used in this study was taken from a body of water into which various unregulated and untreated drainage discharges are discharged, located in the urban area of Mineral de la Reforma, Hidalgo, Mexico (N 20°6′33.790″, W 98°43′29.680″), where in the months of August to January the temperature can range between 4 and 24 °C, and in the period where the experiment was carried out, the range was 17.5 to 21.3 °C. The studies were carried out in batches, at a microcosm scale; the plants were placed in glass containers and designated a protected area under shade that allowed adequate exposure to air and sunlight.

2.2. Construction of the AFI

The AFI was constructed using elbows and 0.5-inch PVC piping to form a 12 cm × 36 cm outer frame, over which plastic mesh, jute fiber mesh, and plastic bottles were installed (Figure 1A). The basal part of the plant rests between the plastic mesh and the jute mesh. The first mesh serves as a support for the plants, and the second gives a better appearance to the floating structures. The plastic bottles ensure that the structure remains floating and are located under the PVC structure [30]. The aerial parts of the plants protrude above the plant fiber, and the roots extend below the floating structure and towards the bottom of the body of water.

(**A**) (**B**)

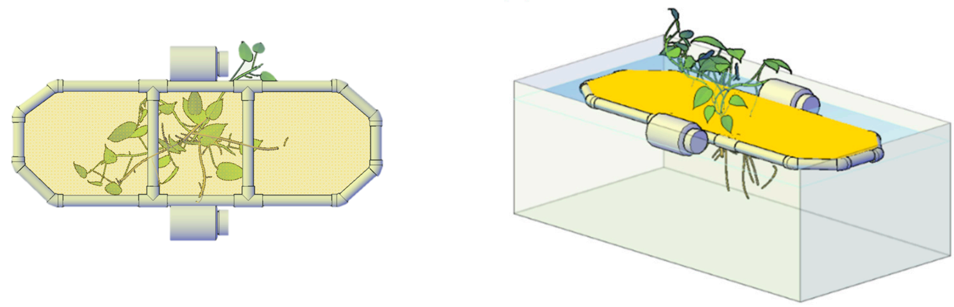

Figure 1. (**A**) AFI structure and (**B**) phytoremediation bioassays.

2.3. Selection of Macrophytes

The aquatic macrophytes *E. crassipes*, *P. stratiotes*, and *S. molesta* were collected from a body of water in Veracruz, Mexico (N 18°47′ 20.360″, W 97°11″ 50.751). Healthy plants were selected, with a length of 10 ± 3 cm above the roots and an average individual weight of 20 ± 5 g.

2.4. Operation of Phytoremediation Bioassays

This research was performed in UW 20 L batch glass cells without water flow. There were three plants in each glass cell. The experiments were carried out in a laboratory with sufficient sunlight under a natural day–night regime, that is, 12 h of light and 12 h of darkness. The experiments were carried out from 1 August to 15 December 2023. The plants were acclimatized to the glass cells with tap water five days before the start of the study [28]. For the phytoremediation bioassays, the hydraulic residence time was 15 days. Sampling was carried out at intervals of two days. The collected wastewater samples were transferred to the laboratory for water quality analysis. Readings were recorded in triplicate, and the average results obtained were expressed as means ± standard deviations. The GraphPad PRISM® package (version 8.0.1) was used for the analysis of variance (ANOVA) to evaluate the significance of differences, and the Student's *t*-test was also used. Furthermore, a p-value less than 0.05 was considered statistically significant.

2.5. Physicochemical Characterization of UW

Determinations of temperature, turbidity, pH, DO, EC, and TDS were made with the portable multiparameter analyzer HANNA HI 9829. NO_3-N concentrations were measured with a nitrate test kit (Hanna Instruments HI97728B, Romania); for PO_4^{3}-P, a phosphate colorimeter (Hanna Instruments HI713, USA) was used. The determinations of TOC, IC, and TN were quantified using a Shimadzu-brand total organic carbon analyzer (32442). The 5220 D colorimetric method was used [31–33] to determine the COD.

2.6. Analysis of Water Samples

The collection, conservation, and physicochemical analysis of the samples followed the procedures established in the Standard Methods [31]. The concentrations of DO, TDS, EC, COD, $PO_4{}^{3}$-P, TOC, NT, and NO_3-N, as well as pH and turbidity, were evaluated during the retention time. Subsequently, removal efficiencies (*E*) were calculated using Equation (1).

$$E(\%) = \left(\frac{C_0 - c_t}{C_0}\right) \times 100 \quad (1)$$

where C_0 is the initial concentration of a contaminant (mg/L) and Ct is the final concentration of the same contaminant (mg/L).

2.7. Phytoaccumulation Evaluation

The ability of macrophytes to absorb and accumulate potentially toxic elements from water can be described by the bioconcentration factor (BCF). The BCF is determined by the relationship between the concentrations of toxic elements in the dry mass of the roots of macrophytes and in the sediment generated in the water. The translocation factor (TF) is defined as the ability of macrophytes to transfer potentially toxic elements from the roots to the upper parts of the plant. In this study, the TF value was estimated as the ratio between the concentrations of potentially toxic elements (NO_3-N and $PO_4{}^{3}$-P) in the leaves of macrophytes and in the roots. BCF and TF values were calculated using the following equations [34]:

$$BCF = \frac{C_{roots}}{C_{water}} \quad (2)$$

$$TF = \frac{C_{leaves}}{C_{roots}} \quad (3)$$

where C_{roots} and C_{leaves} represent the concentrations of NO_3-N and $PO_4{}^{3}$-P in the macrophyte tissues (mg/g) and C_{water} is the concentration of NO_3-N and $PO_4{}^{3}$-P in the UW (mg/L).

Bioaccumulation can be evaluated using BCFs and the following categories: 0.001 < BCF < 0.01, very weak absorption; 0.01 < BCF < 0.1, weak absorption; 0.1 < BCF < 1, intermediate absorption; 1 < BAC < 10, strong absorption; and 10 < BCF < 100, intensive absorption [35]. On the other hand, a TF value >1 indicates a good transfer system and phytoextraction capacity [36]. A BCF >1 and a TF > 1 show phytoextraction capacity, while a BCF >1 and a TF <1 may only show phytostabilization (phytoimmobilization) capacity [35].

To determine the concentration of NO_3-N and $PO_4{}^{3}$-P in roots and leaves, each plant was cut and divided into roots, stems, and leaves; subsequently, these were dried in an oven at 100 ± 5 °C for 4 h. Once the vegetative parts were dry, each part was ground in a mortar and then passed through a 40-mesh mesh [37,38]. The powder obtained was stored in polyethylene bags for the determination of nitrate and phosphate concentrations. For this, 100 mg of sample was weighed, and 20 mL of distilled water at 80 °C was added. The resulting solution was stirred for 30 minutes using a magnetic stirrer. Then, the resulting solution was filtered through borosilicate membrane filters of 0.45 mm porosity [39]. The method used to determine nitrates is based on the reduction that nitrate undergoes to nitrite when passing through a cadmium column. The phosphate method is an adaptation of the ascorbic acid method [40].

2.8. Evaluation of Growth Attributes in Macrophytes Exposed to UW

Each plant was weighed individually before the experiment, and at the end of the hydraulic retention time, a digital scale (SOLI, USS-DBS15-5, IN) was used. The average weight of three plants was taken as the effective fresh plant biomass, and the results were expressed as relative growth rates (RGRs). For this, the logarithmic equation [41] was used to express the relative changes in biomass over a period of experimental time (Equation (4)).

$$RGR\left(gg^{-1}d^{-1}\right) = \left(\frac{ln\ w_t - ln\ w_0}{\Delta t}\right) \quad (4)$$

where W_t and W_0 are estimated fresh plant biomasses and Δt is a 15-day time interval.

To estimate the total chlorophyll content, the leaves of each macrophyte were washed and subsequently crushed in a mortar; 1 g of the crushed sample was taken and mixed with 5 ml of 80% acetone. After grinding the sample, the content was brought to 50 mL by adding more acetone solution and then centrifuged at 10,000 rpm for 30 min (Z 383, Hermle LaborTechnik GmbH). Finally, the supernatant was separated, and the absorbance was taken using a double-beam UV-Vis spectrophotometer (4001/4, Scientific™ GENESYS 20) at 645 and 663 nm, as described by Pérez-Patricio et al. [42] and Kumar et al. [43].

$$Total\ Chlorophyll\left(mgg^{-1}\right) = (8.2 * A_{663}) + (20.2 * A_{645}) \quad (5)$$

where A_{663} and A_{645} are the absorbances at 663 nm and 645 nm, respectively. The spectrophotometer was set to zero using 80% acetone.

3. Results and Discussion

3.1. Physicochemical Characterization of UW

Table 1 shows the average values for the physicochemical characterization of the wastewater. The values were as follows: 1.63 mg/L for DO, 2639.5 µS/cm for EC, 781 mg/L for COD, 29.04 mg/L for PO_4^{3}-P, 170 63 mg/L for TOC, 117 mg/L for NT, 22.6 mg/L for NO_3-N, 7.81 for pH, and 248 FTU for turbidity. Mendoza et al. [44] characterized municipal wastewater from the city of Riohacha in Colombia, where the water had a pH of 7.60 ± 0.16 and the other values were as follows: DO: 1.19 ± 0.79, COD: 355 ± 115, NO_3-N: 1554 ± 1694, and PO_4^{3}-P: 2975 ± 2457. Similar values, such as those for pH, DO, and COD, were found in this research. On the other hand, Kobir et al. [45] sampled urban wastewater in Kushtia and Jhenaidah, Bangladesh. Determinations in the Kushtia municipal area ranged between 0.21 and 1.24 mg/L, 97.33 and 592.34 mg/L, and 431.34 and 849.33 mg/L for DO, COD, and TDS, respectively. In Jhenaidah Municipality, levels ranged between 0.34 and 1.72 mg/L, 55.33 and 491.67 mg/L, and 412.34 and 895.66 mg/L for DO, COD, and TDS, respectively.

Table 1. Physicochemical characterization of the UW.

Parameter	Value
Temperature (°C)	17.4 ± 2.6
pH	7.81 ± 0.71
EC (µs/cm)	2639 ± 14.85
TDS (mg/L)	1319 ± 7.78
DO (mg/L)	1.63 ± 0.04
Turbidity (FNU)	248 ± 31.82
COD (mg/L)	741 ± 4.36
TOC (mg/L)	170 ± 3.73
PO_4^{3}-P (mg/L)	29 ± 0.51
NO_3-N (mg/L)	22 ± 1.06
TN (mg/L)	117 ± 0.45

The temperature range of water considered appropriate for the growth of aquatic plants is 15 to 38 °C. A temperature of 20 °C is considered optimal, and the lowest value that plants can withstand for their development is 6 °C [46]. The water temperature throughout this study was 17.4 ± 2.6 °C, which shows that the phytoremediation process was carried out under adequate conditions and that this factor did not significantly affect the phytoremediation process.

3.2. Monitoring and Evaluation of Bioassays

Table 2 shows the parameters evaluated in the bioassays using *E. crassipes*, *P. stratiotes*, and *S. molesta*.

Table 2. Output values for phytoremediation bioassays.

Parameter	*E. crassipes*	*P. stratiotes*	*S. molesta*
pH	8.3 ± 0.28	8.44 ± 0.02	8.39 ± 0.4
EC (µs/cm)	2943 ± 9.65	2885 ± 15.6	3007 ± 21.9
TDS (mg/L)	1479 ± 14.10	1417.5 ± 2.8	1463.0 ± 3.5
DO (mg/L)	1.6 ± 0.3	0.5 ± 0.0035	0.18 ± 0.008
Turbidity (FNU)	75.14 ± 24.7	94.24 ± 28.3	186.0 ± 29
COD (mg/L)	231.19 ± 26.8	285.28 ± 61	315.67 ± 26
TOC (mg/L)	70.21 ± 4.5	55.08 ± 2.7	84.92 ± 4.5
PO_4^{3}-P (mg/L)	8.99 ± 0.51	8.99 ± 0.3	27.26 ± 0.7
NO_3-N (mg/L)	9.61 ± 0.8	16.54 ± 1.5	20.28 ± 0.4
TN (mg/L)	69.14 ± 0.5	67.86 ± 1.3	81.67 ± 3.5

For the bioassays where only *E. crassipes* was used, there was an increasing trend in some parameters; the increase was 6.3%, 11.5%, and 12.1% for pH, EC, and TDS, respectively. However, the DO decreased by 1.8%. For the tests using *P. Stratiotes* (Table 2), it was observed that the pH showed an increase of 8.1%, while EC increased by 9.3% and TDS increased by 7.5%. On the contrary, DO values decreased by 69.3%. In the case of the bioassays with *S. molesta*, the pH values increased by 7.4%, EC by 13.9%, and TDS by 10.9%; however, DO decreased by 89% (Table 2).

Alkaline pH is favorable for wastewater treatment by aquatic plants [44]. During the phytoremediation bioassays with the three macrophytes, an increase in pH from 7.81 to 8.4 occurred, indicating that the bioassays underwent alkalinization. In general, the experiment was carried out at an optimal pH (7–8) for nutrient absorption and the biochemical reactions of living organisms. The change in pH may have been due to the consumption of CO_2 resulting from the photosynthetic activities of the macrophytes; on the other hand, in the rhizospheres of plants, an imbalance may occur due to the absorption of cations and anions, which can change the pH [44]. In the phytoremediation process with aquatic plants, various mechanisms operate, the main ones being sedimentation and filtration of contaminants [46]. Due to these mechanics and alkalinization, an increase in TDS and EC may occur. For this research, in the three phytoremediation bioassays, an increase in TDS and EC occurred, which may have been due to the generation of carbonates and sediment generation.

DO dynamics for an aquatic system are often complex and considered an essential component for assessing water quality. In this investigation, there were low DO concentrations due to the concentration in the influent and probably because aeration in the systems was not successful. The dissolved oxygen was higher before the treatment and tended to decrease due to various factors; the most relevant in this context was the decomposition of the plants; this reduces the amount of dissolved oxygen, and therefore it could also have affected the performance of the treatment [47]. Similar observations were presented by Mera and García [48], who treated urban wastewater with a pH of 5.5 and an EC of 156 S/cm; for the treatment with *E. crassipes*, the treatment time was nine days, the pH increased to 8.17, and the EC was 256.4 S/cm.

3.3. Removal Efficiency of Physicochemical Parameters

The removal efficiencies for turbidity, COD, TOC, PO_4^{3}-P, NO_3-N, and NT can be seen in Table 3.

For the bioassays with *E. crassipes*, efficiencies greater than 40% were achieved, and for COD (68.8%), PO_4^{3}-P (69.7%), and turbidity (69.7%), the efficiencies were close to 70%. The results obtained for the pollutant removal efficiencies by *P. stratiotes* (Table 3) were mostly higher than 40%, except for nitrate removal, with only 24.8% removed. The highest elimination achieved was for PO_4^{3}-P, with a value of 67.6%. The bioassays with *S. molesta* (Table 3) were diverse; the lowest efficiency achieved was 7.8% for NO_3-N, while the highest removal was 57.4% for COD. In comparison with the other bioassays, significant differences were noted ($p < 0.05$). As can be seen in the present study, the three macrophytes can eliminate various contaminants. Still, it is *E. crassipes* that showed a more extraordinary absorption capacity than the other aquatic plants. Mendoza et al. [44] evaluated the potential of *E. crassipes* in the treatment of municipal wastewater over 14 days. The wastewater had initial concentrations of 355 ± 155 mg/L of COD, 2975 ± 2457 mg/L of $PO_4{}^3$-P, and 1,554 ± 1694 mg/L of NO_3-N. For the study, 40 liters were used per experiment, and elimination efficiencies of 74.8%, 44.4%, and 21.1% were achieved for COD, $PO_4{}^3$-P, and NO_3-N, respectively. These results are like those presented in our research; however, the other group achieved higher removals, which can be attributed to the fact that more plants were used in their research, with up to 16 plants per experiment.

Table 3. Removal efficiency of phytoremediation bioassays.

E (%)	*E. crassipes*	*P. stratiotes*	*S. molesta*
Turbidity	69.7 ± 1.2	62 ± 7.0	25 ± 3.1
COD	68.8 ± 5.0	61.5 ± 14.1	57.4 ± 9.5
TOC	58.7 ± 0.1	59 ± 1.2	50.5 ± 1.6
$PO_4{}^3$-P	69.4 ± 1. 4	67.6 ± 0.6	6.0 ± 1.8
NO_3-N	56.3 ± 0.10	24.8 ± 0.2	7.8 ± 0.7
TN	40.9 ± 0.9	42.2 ± 2.4	30.2 ± 2.8

Cárdenas et al. [49] studied the purification capacity of *Pistia stratiotes* L. in the treatment of synthetic waters, and the hydraulic retention time was nine days. The results showed that there was an increase in pH (5.4 to 6.2) and DO (4.78 to 12.09 mg/L of O_2). The COD content was 214.8 mg/L, reaching an elimination of 40.8%. On the other hand, Haydar et al. [50] evaluated the phytoremediation potential of *P. stratiotes* in the treatment of municipal wastewater; the hydraulic retention time was eight days. The initial concentrations were 451 ± 452 mg/L of COD, 1.4 ± 0.2 mg/L of phosphorus (P), and 37.5 ± 1.85 mg/L of Total Kjeldahl Nitrogen (TKN), and removal efficiencies of 80% for COD, 88% for P, and 82% for TKN were achieved.

Mustafa and Hayder [51] evaluated the performance of *S. molesta* in phytoremediation of domestic wastewater samples treated for 14 days, with a retention time of 24 h; the efficiencies were 97.7%, 99.7%, 99%, and 90.6% for turbidity, phosphate, ammoniacal nitrogen, and nitrate, respectively. In this research, a hydroponic system was used, which kept the water in recirculation and under constant aeration, which could have allowed greater removal of the macrophyte. On the other hand, Ng and Chan [52] investigated the phytoremediation performance of *S. molesta* in an AFI; for this, they used a palm oil manufacturing effluent, and the hydraulic retention time was 16 days. According to the obtained results, turbidity decreased from 7.56 NTU to 0.94 NTU in just two days. The initial concentration of phosphate was 3.5 mg/L, eliminating 95%; for COD, the concentration was 9 to 64 mg/L COD, reaching an elimination of 39%. What this suggests to us is that the conditions in which the phytoremediation process is developed using *S. molesta* considerably affect the performance and that recirculation and aeration passively benefit the elimination of contaminants.

3.4. Phytoaccumulation Evaluation

The BCF and TF values for nitrates and phosphates in the three macrophytes are represented in Figure 2. The BCF values (Figure 2A) referring to nitrates for *P. Stratiotes* and *S. molesta* were below 1; the highest value was 2.89 ± 0.23 mg/g with *E. crassipes*. The BCF values for phosphates for *E. crassipes* were 4.88 ± 0.08 mg/g, and for *P. stratiotes*, they were 3.48 ± 0.20 mg/g, which exceeded the value of 1, suggesting strong absorption by these two macrophytes. *S. molesta* showed an intermediate absorption (0.1 < BCF < 1) with 0.15 ± 0.03 mg/g.

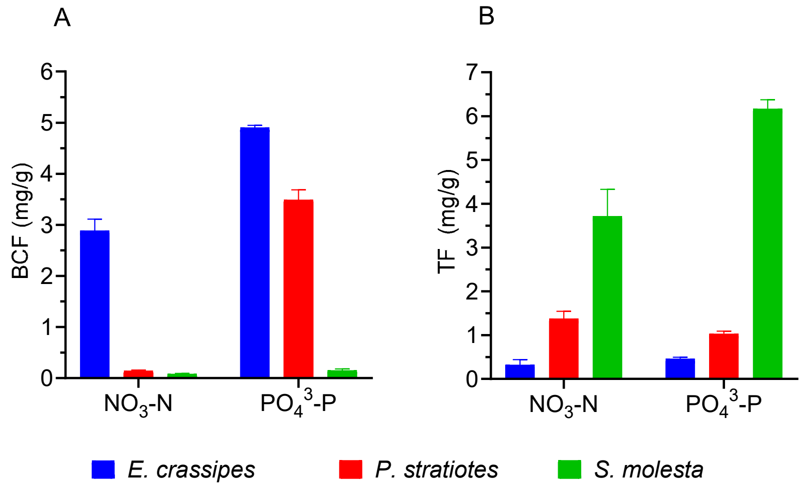

Figure 2. (**A**) BCF values and (**B**) TF values for the three macrophytes.

The highest TF values (Figure 2B) were reached by *S. molesta* with 3.72 ± 0.61 mg/g and 6.17 ± 0.21 mg/g for nitrates and phosphates, respectively. *P. stratiotes* reached values of 1.38 ± 0.17 mg/g and 1.03 ± 0.06 mg/g for nitrates and phosphates, respectively. These results suggest a high translocation capacity from the roots to the leaves. On the other hand, *E. crassipes* reached values of 0.32 ± 0.12 mg/g and 0.46 ± 0.03 mg/g for nitrates and phosphates, respectively. A TF < 1 indicates ineffective translocation.

The BCF results showed that the three macrophytes have a higher affinity for phosphates, which are considered important nutrients for vegetative reproduction. Vegetative growth is the main resource for the assimilation of PO_4^{3}-P. During the growth stages, plants need PO_4^{3}-P to develop their biomass [46], such that aquatic plants are more susceptible to its assimilation.

The results suggest that *S. molesta* and *P. stratiotes* have a high translocation capacity for toxic compounds and a high phytoextraction capacity. On the other hand, *E. crassipes* has a low translocation transfer; this macrophyte has a tolerance strategy for toxic compounds with a high retention capacity in the roots. This may be due to its long and dense roots, which are responsible for absorbing nutrients and therefore act to intercept and absorb suspended particles [50], which suggests that the mechanism of contaminant retention is phytostabilization.

Wibowo et al. [53] investigated the bioaccumulation of *E. crassipes* and *P. Stratiotes* using water contaminated with Fe and Mn; both plants showed that the bioaccumulation of heavy metals is achieved in greater proportions in the roots: the BCF in the root of *E. crassipes* was 0.075 mg/g, and in the root of *P. stratiotes,* it was 0.070 mg/g. The TF value for *E. crassipes* was 0.85 mg/g, and that for *P. stratiotes* was 0.90 mg/g, indicating that the plants are hyperaccumulator plants. One of the factors that could have affected the higher concentration of heavy metals in the roots may have been that the roots were closer to the source of metal ions, and, as in the aforementioned study, this suggests that the mechanism used by these plants is phytostabilization.

Li et al. [54] studied the mechanisms underlying the uptake, accumulation, and translocation of organophosphate esters and brominated flame retardants in a typically polluted river using free water hyacinth. Passive absorption by the roots was the dominant route, with a higher concentration of contaminants in the roots and the possibility of translocation to the leaves. Translocation in water hyacinth also showed a close association with its degree of bromination, but its accumulation in the roots showed anomalies, indicating possible transformations. Plant biomass showed significant effects on root accumulation and translocations in water hyacinth. Lao et al. [28] investigated accumulation and transformation in *E. crassipes* through a series of hydroponic experiments to measure phosphate contents.

It was discovered that phosphates can enter the roots of plants through passive diffusion pathways, but they can also return to a solution when concentration gradients exist.

3.5. Effects of UW on Macrophyte Attributes

The bioavailability of nutrients within water is the most important factor affecting plant growth [55]. In the phytoremediation experiments with the three macrophytes, the UW contained significant nutrients that promote growth. However, different plant growth parameters, such as total fresh biomass, total chlorophyll content, and RGR, were significantly affected ($p < 0.05$) by the effluent concentration and its nutrient composition. The total chlorophyll content for *E. crassipes* was 45.97 ± 0.8 mgg^{-1} fwt, and for *P. stratiotes* it was 2.94 ± 0.1 mgg^{-1} fwt. For *S. molesta*, it was 2.20 ± 2.3 mgg^{-1} fwt (Figure 3A), and the RGR for *E. crassipes* was 0.00024 gg^{-1}d^{-1}; however, for *P. stratiotes*, there was a loss of biomass reflected in the decrease of 0.0046 gg^{-1}d^{-1}, and for *S. molesta* the biomass similarly decreased by 0.018 gg^{-1}d^{-1} (Figure 3B). These phenomena may have been due to the composition of the UW, which tended to have an adequate nutrient content for *E. crassipes* and to be toxic for *P. stratiotes* and *S. molesta*; this medium is unfavorable, since it affects the growth of and causes necrosis in these plants.

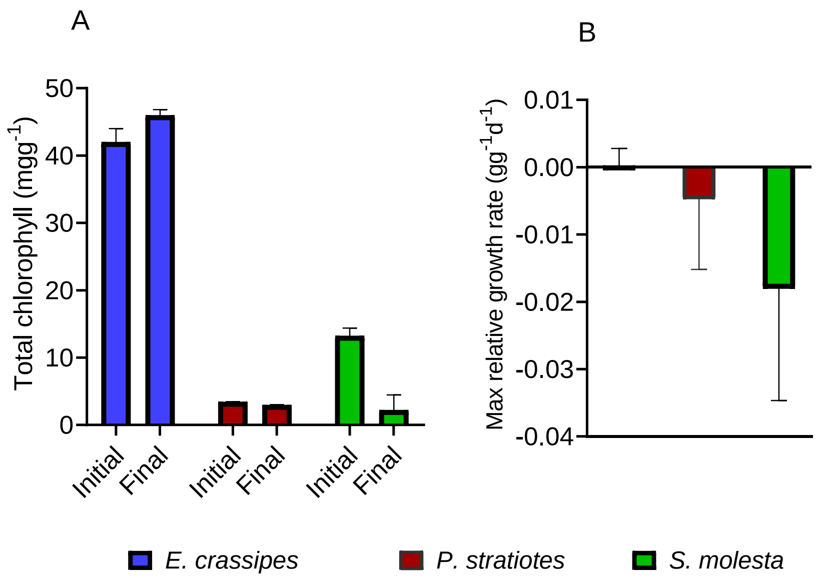

Figure 3. (**A**) Total chlorophyll and (**B**) RGR values for the three macrophytes.

The increase in total chlorophyll content for *E. crassipes* was like that presented by Kumar et al. [56], who reported that the maximum total chlorophyll content for *E. crassipes* was 1.50 ± 0.3 mgg^{-1}fwt. It increased by 50% after 60 days of phytoremediation with wastewater from the manufacture of pulp and paper. Photosynthetic pigments, such as chlorophyll, in green plants are important components of the photosynthesis system. A significant change in the number of pigments can have adverse consequences for the entire metabolism of the plant through the degradation of membrane lipids and reactive oxygen species, which implies that plants reduce their nutrient intake [41]. This research shows that *P. stratiotes* and *S. molesta* plants exhibited a decrease in chlorophyll production, which was related to a low removal of contaminants due to a decrease in nutrient intake.

Likewise, exposure to high concentrations of contaminants directly affects the growth of aquatic macrophytes; they can have harmful effects, such as loss of biomass, wilting, and leaf chlorosis, which leads to the RGR being directly affected. Singh et al. [57] studied the effectiveness of *E. crassipes* for the reduction of heavy metals from highly toxic effluents of the glass industry. The evaluation period was 40 days, and the total chlorophyll content was 3.53 ± 0.11 mgg^{-1}fwt and the RGR was 0.0026 gg^{-1}d^{-1}, showing that there was an increase in biomass and an increase in chlorophyll generation.

The nutrients present in UW act as food for plants and are essential for their growth. The growth rate is directly related to the plant's nutrient removal capacity. Aquatic macrophytes have the potential to double their biomass within 7 to 15 days [46], indicating that RGR needs to increase. An increase in RGR is directly related to nutrient removal capacity.

The term "hormesis" refers to the increase in chlorophyll in certain aquatic plants such as *E. crassipes*. Through this mechanism, they control an alteration of homeostasis due to the induction of environmental stress. It is one of the defense mechanisms that are stimulated in adverse environmental conditions [58]. Due to the hormesis mechanism and the increase in RGR, it is evident that *E. crassipes* can adapt to toxic environments such as UW, and, in the same way, a greater removal of contaminants by this plant is reflected.

4. Conclusions

The use of an AFI with floating macrophytes proved to be effective for the removal of residual nutrients present in the UW utilized in this study. The selection of suitable plants can help to create a system with greater removal capacity. Of the three macrophytes used, it was observed that *E. crassipes* surpassed *P. stratiotes* and *S. molesta* in removal efficiency with respect to turbidity (69.7%), COD (68.8%), PO_4^{3}-P (69.4%), and NO_3-N (56.3%), and only in the elimination of TOC (59%) and NT (42.2%) did *P. stratiotes* achieve the highest efficiency. In the uptake of nitrates and phosphates by the three macrophytes, greater assimilation of phosphates was shown. These nutrients tend to be ingested by roots through passive diffusion from a solution and transported to the leaves. However, for *E. crassipes*, there was greater accumulation in the roots, which could indicate slower translocation. However, *P. stratiotes* and *S. molesta* showed greater accumulation in the leaves, indicating a higher translocation speed. Biomass generation and chlorophyll production in plants were affected during the study period, with decreases in both *P. stratiotes* and *S. molesta*. On the other hand, for *E. crassipes*, the RGR was 0.00024 $gg^{-1}d^{-1}$, and the total chlorophyll production increased by 9.46%, which demonstrates its greater capacity for adaptation and nutrient sequestration. Of the three macrophytes used, based on the parameters evaluated, *Eichhornia crassipes* has the greatest potential. The impressive overall performance of *Eichhornia crassipes* makes it the best candidate for AFI applications aimed at nutrient remediation. However, this study investigated only three floating macrophytes, and it would be necessary to expand this research by including different plants. Thus, its applicability in other regions may be limited. Future studies should focus on investigating AFIs in field experiments to understand their performance under dynamic conditions more broadly. Furthermore, performing biomass nutrient analyses for large-scale AFIs will help gain a deeper and more appropriate understanding to optimize the performance of AFIs.

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