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Nutrient removal by Eichhornia crassipes and its impact on the water column and its biomass production

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Article

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Nutrient removal by *Eichhornia crassipes* and its impact on the water column and its biomass production.

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Abstract

High nutrient concentrations in water bodies cause eutrophication. This promotes the presence, development, and uncontrolled growth of invasive species, such as *Eichhornia crassipes*. Its use for bioremediation in confined environments is being considered as a biomass utilization strategy. This is due to its potential for removing nutrients from the water column. To test this, a mesocosm bioassay was conducted with two treatments: a control and one enriched with commercial fertilizer. Physicochemical parameters (dissolved oxygen, pH, and electrical conductivity) and the dynamics of nutrient concentrations (P and N) were evaluated in each treatment. Ten *E. crassipes* organisms were placed in each mesocosm. The morphology of three randomly selected individuals was measured, recording length and maximum diameter of the petiole, length and width of the leaf blade, and root length. The results show the plant's removal potential as nutrient concentrations decreased: NT by 11.42% and PT by 33.14% throughout the mesocosm. An improvement in physicochemical conditions was also observed, with dissolved oxygen increasing from 0.8022 mgL-1 to 2.4388 mgL-1 and pH from

6.5 to 7.5. This demonstrates the plant's removal efficiency, its buffering capacity of physicochemical parameters in the water column, and its ability to keep nutrients suspended in the water column.

Keywords: *Eichhornia crassipes,* wáter hyacinth, phytoremediation, nutrients.

1. Introduction

Freshwater environments are biodiversity hotspots [30,35], and they also provide ecosystem services (such as flood prevention, aquifer recharge, carbon sinks, and regulation of biogeochemical cycles, etc.) However, the increasing demand for water as a resource in various anthropogenic activities (e.g., agriculture) is degrading water resources [61, 39].

Within agricultural activities, the excessive use of fertilizers composed of limiting nutrients (Phosphorus and Nitrogen) is where the greatest contribution of these pollutants to these environments occurs [34, 38], followed by input from non-point sources [46, 27, 59, 55; 33], leading these ecosystems to eutrophic conditions, with direct and indirect consequences for the surrounding biota [15; 6; 9; 7; 63]. In turn, high nutrient loads interact synergistically with rising temperatures to exert toxic effects on pelagic species [67].

Among the toxic effects, phosphorus (P) in the form of phosphate (PO4+) and nitrogen (N) in the form of ammonium (NH4+), nitrite (NO2-), and nitrate (NO3-) stand out [21, 22, 58, 14]. Phosphorus compounds promote bone malformations, oxidative stress, DNA damage, and teratogenicity [19]. On the other hand, nitrogen and its chemical forms can act as endocrine disruptors for different organisms [48,

20, 29, 60, 28]. In turn, it can modify the structure of the microbial community by affecting its life cycles [8, 16, 10, 64]. Because of this, strategies have been implemented to mitigate this problem and improve water quality, such as phytoremediation [16, 24, 36, 45].

One species with phytoremediation potential is the free-floating macrophyte *Eichhornia crassipes* (water hyacinth), due to its physicochemical buffering capacity for dissolved oxygen, pH, and turbidity, among other factors, and its ability to remove various pollutants (heavy metals, emerging contaminants, etc.) [62, 50, 47]. Therefore, this study was carried out with the objective of determining the nutrient removal efficiency performed by the macrophyte E. crassipes thanks to its resource utilization characteristics, linked to its reallocation capacity, which is proposed as a suitable biological model for bioremediation purposes, thus reducing the impact generated by eutrophication, the dynamics of nutrient distribution in the water column, in turn controlling the population density of this species through its utilization.

2. Material and methods

2.1. Collect and acclimatization of *Eichhornia crassipes*

We collected E. crassipes plants in accordance with institutional and national policies from the canals of the Cuemanco dock, Xochimilco, Mexico City (19°17'58.50"N, 99°6'2.09"W), with SEMARNAT collection permission (voucher SGPA/DGVS/00962/22). We selected them by excluding those showing organ damage, chlorosis, necrosis, or turgor loss. We collected the samples manually with a scoop net and stored them in a water-filled cooler for transport to the

laboratory. Claudio Dario Rueda Barrios identified one of these collected organisms and stored it in the IZTA herbarium at the Faculty of Higher Studies Iztacala (Voucher: FESI/HI/008/2026; ID:29042).

In the laboratory, the specimens were first washed with tap water to separate plant organisms from other organisms and macroinvertebrates. Subsequently, a second root wash was performed to remove all organic matter and sediments adhering to the roots. The specimens were then placed in a 250 L container containing a maintenance solution for one week, representing their acclimatization period prior to the bioassay.

2.2. Mesocosm for bioassay

The mesocosms were constructed using polypropylene containers 1 m high and with a surface area of 0.8 m^2, which were then filled to 85% capacity with previously dechlorinated tap water. These were placed outdoors at the Unidad de Investigación Interdiciplinaria en Ciencias de la Salud y Enseñanza (UIICSE), at the Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México (UNAM).

2.3. Nutrients remotion

For the bioassay, the mesocosm surfaces were covered with 10 individuals of the macrophyte. This was carried out over a period of 15 days according to Rezania et al. [42]. Two treatments were performed: one enriched with a nutrient solution (commercial fertilizer: Nutrient Solution for Berries, Solution A, Hydro Environment) with a nutrient concentration of 56.76 mg L^{-1} of total nitrogen (TN) and 45.80 mg

96 L^{-1} of total phosphorus (TP), according to the product label; the other treatment
97 was considered a control without nutrient enrichment. Each treatment was carried
98 out in triplicate.

99 Physicochemical parameters dissolved oxygen (DO), temperature, pH, salinity, and
100 electrical conductivity (EC) were monitored at three different depths (0.1, 0.4, and
101 0.7 m) using a HANNA HI98194 multiparameter probe. Water samples were
102 collected from these depths using a vacuum system through a collection tube.
103 Nitrite (NO2-), nitrate (NO3-), and ammonium (NH4+) were quantified by
104 spectrophotometry according to the methods of Ramírez and Mendoza-Cantú [41],
105 Álvarez [4], and Solorzano [52], respectively. Phosphate (PO4+) was quantified by
106 colorimetry (using a HANNA HI717 checker colorimeter) to monitor its changes
107 during the experimental period.

108 The morphometry of *E. crassipes* individuals was monitored by measuring the
109 maximum petiole length and diameter (PL and PD, respectively), total leaf blade
110 length and width (LBL and WBL, respectively), and root length (RL). These
111 measurements were taken every two days during the bioassay period on three
112 randomly selected organisms from each treatment.

113 In turn, biomass metrics were calculated according to López et al. [31]. For this
114 purpose, five *E. crassipes* individuals were randomly selected from each
115 mesocosm at the beginning and end of each treatment, stored in Kraft paper
116 envelopes, and left to dry for three days before being dissected to separate the
117 root from the shoot of the plant. The dry biomass samples were then weighed

using a granular balance Triple Beam. Once the corresponding weights were obtained, the relative growth rate was calculated (equation 1),

$$(1)\ Relative\ Growth\ Rate = \frac{weight\ final - weight\ initial}{Weigh\ initial}$$

and the shoot:root ratio was later calculated (equation 2).

$$(2)\ ratio\ shoot/root\ \frac{shoot\ wight}{root\ weight}$$

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) was performed to compare treatments using morphometric data and relative growth rates. The physicochemical parameters obtained were analyzed using a two-way ANOVA with treatments and depths as the two variables to be evaluated, in SigmaPlot 11.0 software.

A canonical correspondence analysis (CCA) was used to assess the relationship between plant morphometrics and water physicochemical parameters. CANOCO for Windows 4.5 was used for this analysis.

3. Results

3.1. Dynamics of physicochemical parameters

According to the graphs of physicochemical parameters (Fig. 1), both treatments showed a trend toward alkalinization. At the end of the bioassay (day 14), the control treatment reached a maximum pH of 8.3, while the nutrient-enriched treatment had a pH of 8.1. In the nutrient-enriched treatment, DO concentration increased markedly during the bioassay, reaching 3.4 mg/l at the end (ranging from 0.8 to 3.4 mg/l). Both treatments showed similar temperature values, reflecting

their correlation with ambient temperature. The greatest differences were observed in salinity and EC, which increased in the nutrient-enriched treatment due to dissolved nutrients; the highest values (Fig. 2) were 2761 ppm and 6070.6 μS/cm², respectively.

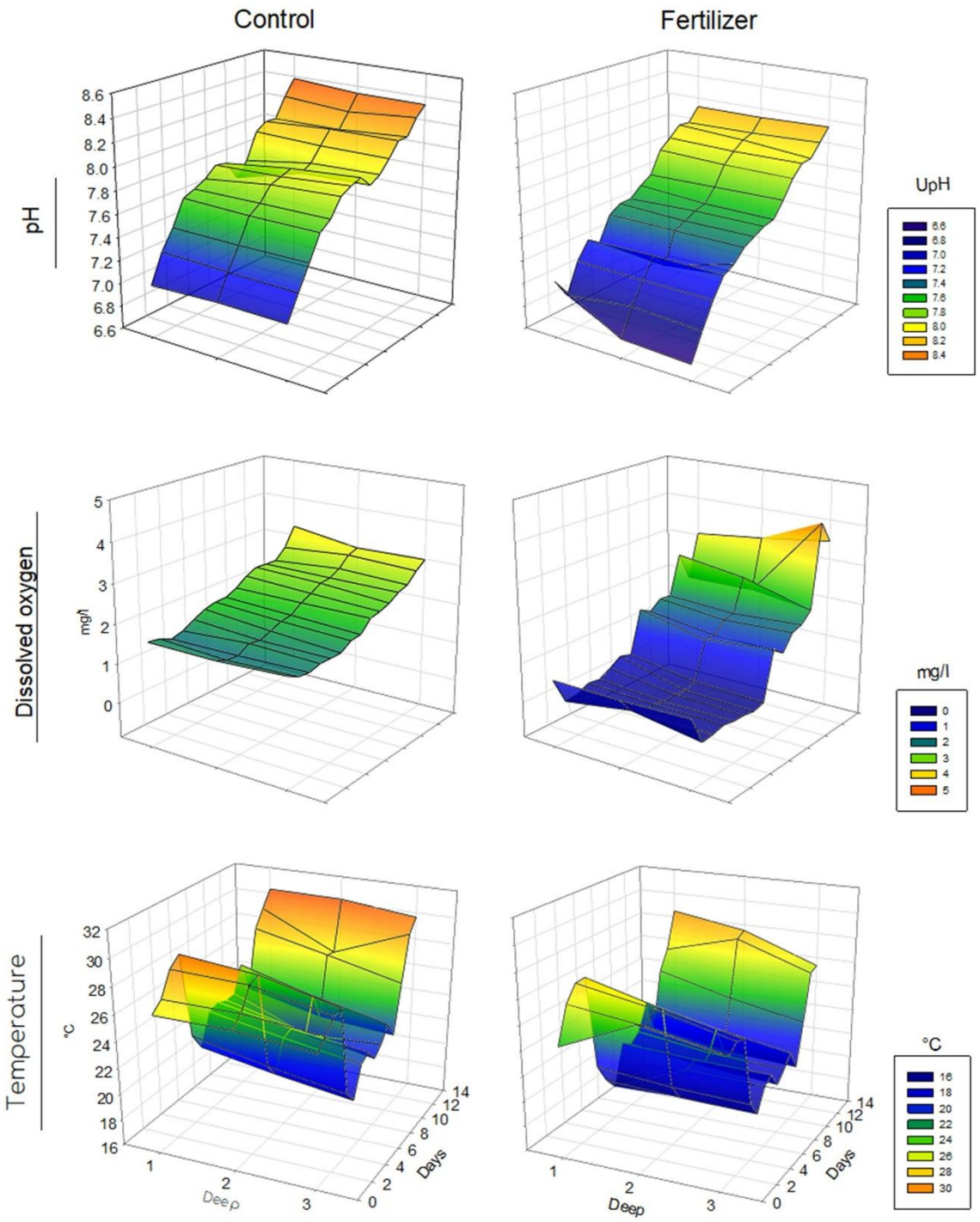


Figure 1: Physicochemical parameters (pH, DO, and temperature) during the bioassay.

143

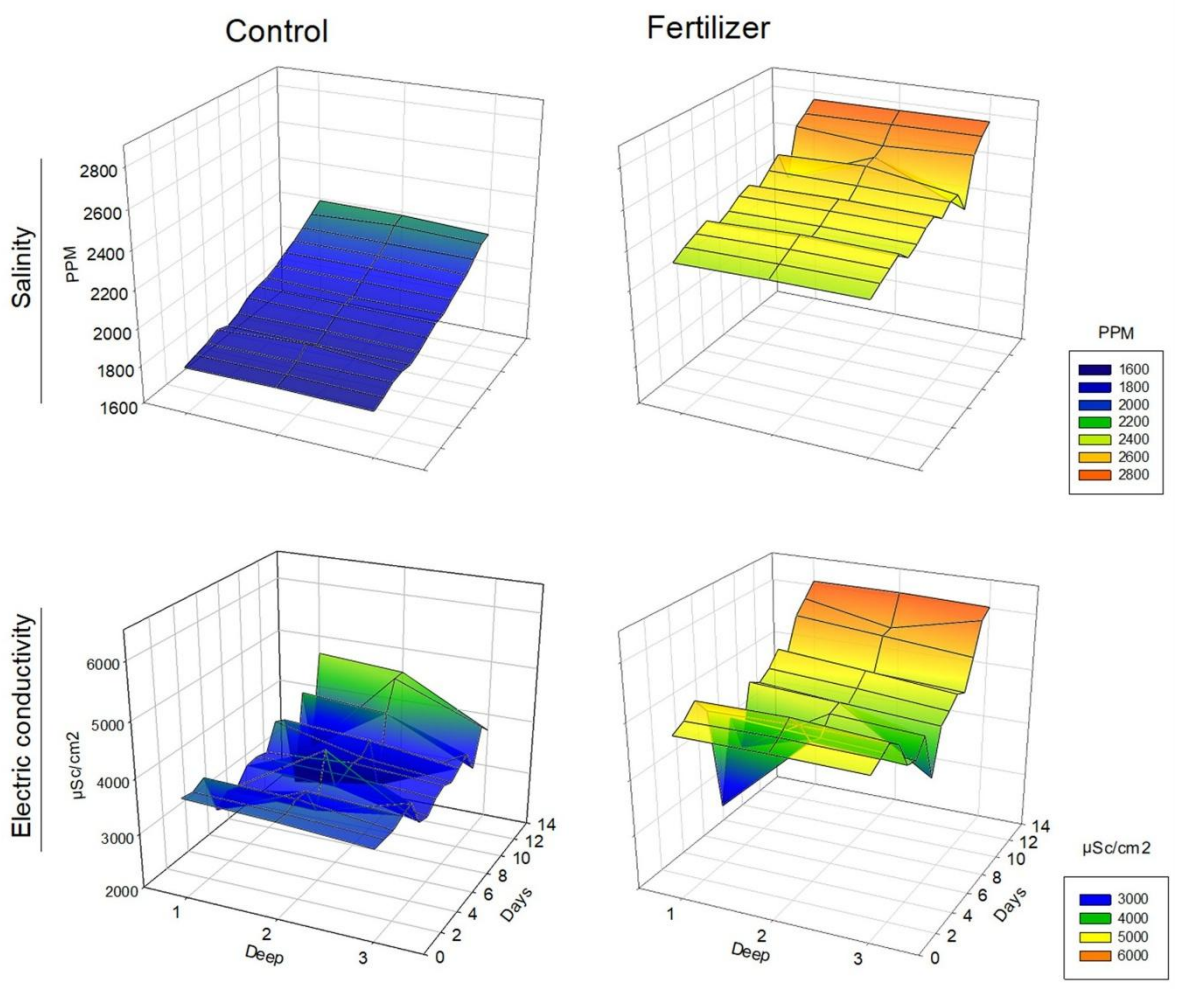


Figure 2: Physicochemical parameters (salinity and EC) during the bioassay.

144

145 **3.2. Nutrients remotion**

146 The greatest nutrient removal (Fig. 3) was reported at depth I (0.1 m), where there
147 was a decrease in nitrogen compounds NH4+ and NO2-, reaching 0.913 mg/l
148 (3.007%) and 1.164 mg/l, respectively, on day 14. Conversely, the greatest removal
149 of NO3-, at 25.153 mg/l (46.061%), occurred at depth III (0.7 m). The greatest
150 decrease in phosphorus levels was observed at depth II, with a removal of 16.466
151 mg/l (35.95%) on day 14.

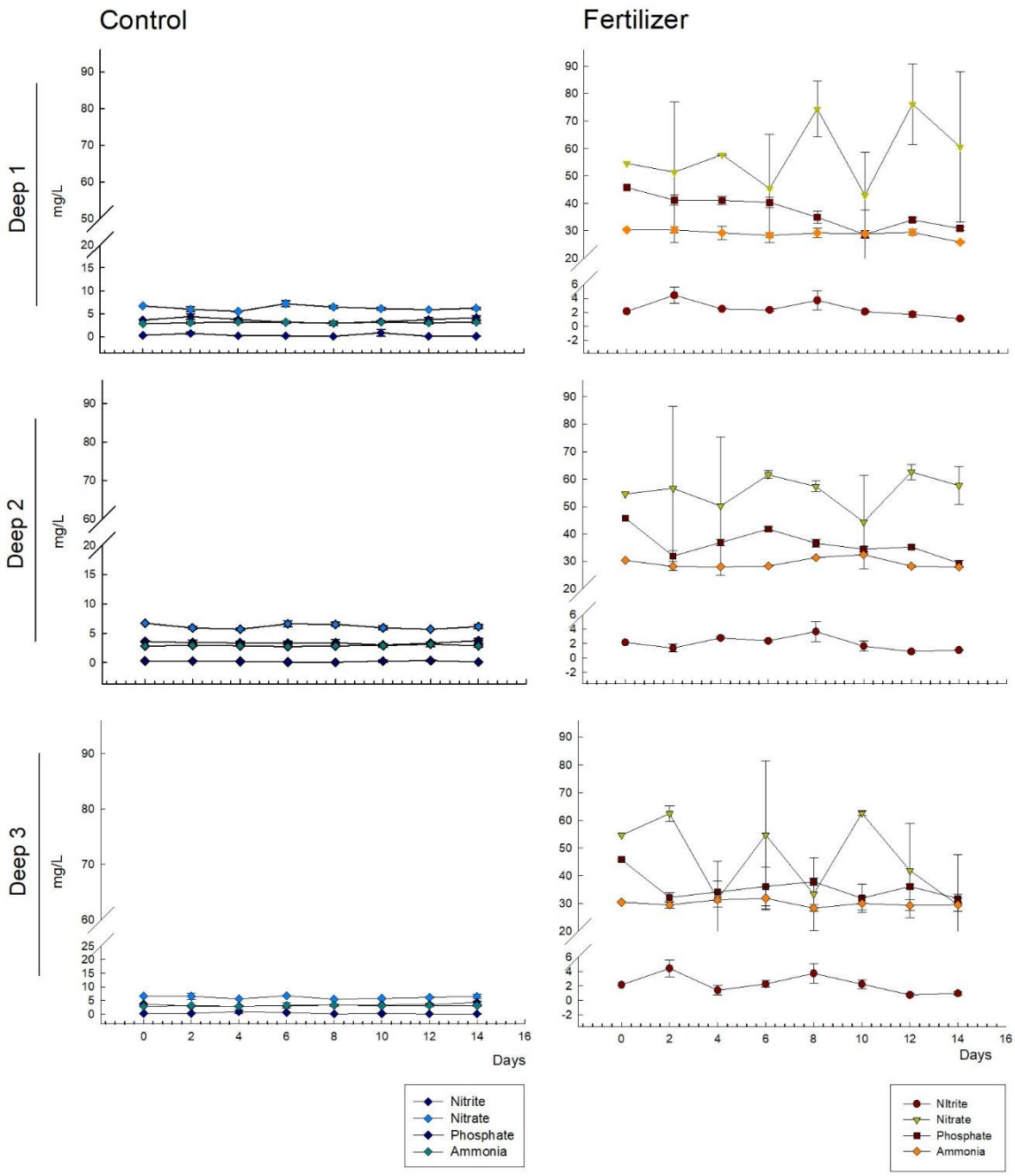


Figure 3: Nutrient variation (NO_2^-, NO_3^-, NH_4^+, PO_4^+) during the bioassay period.

152

153 **3.3. Relationship between physicochemical parameters and**

154 **morphometry of *E. crassipes***

Regarding the correlation between variables for the control treatment, the CCA (Fig. 4A) shows a correlation (with a representativeness of 93.6% of the X and Y axes) between salinity, pH, and dissolved oxygen with root length, while the remaining morphometric variables do not have a correlation with any of these parameters. On the contrary, the CCA of the nutrient-enriched treatment (Fig. 4B) shows a different correlation trend (with a representativeness of 95.7% of the X and Y axes), with phosphate, nitrite, and ammonium being the ones that most influenced foliar morphometric variables (mainly LBL and PD), while the plant morphometric variables did not have any correlation.

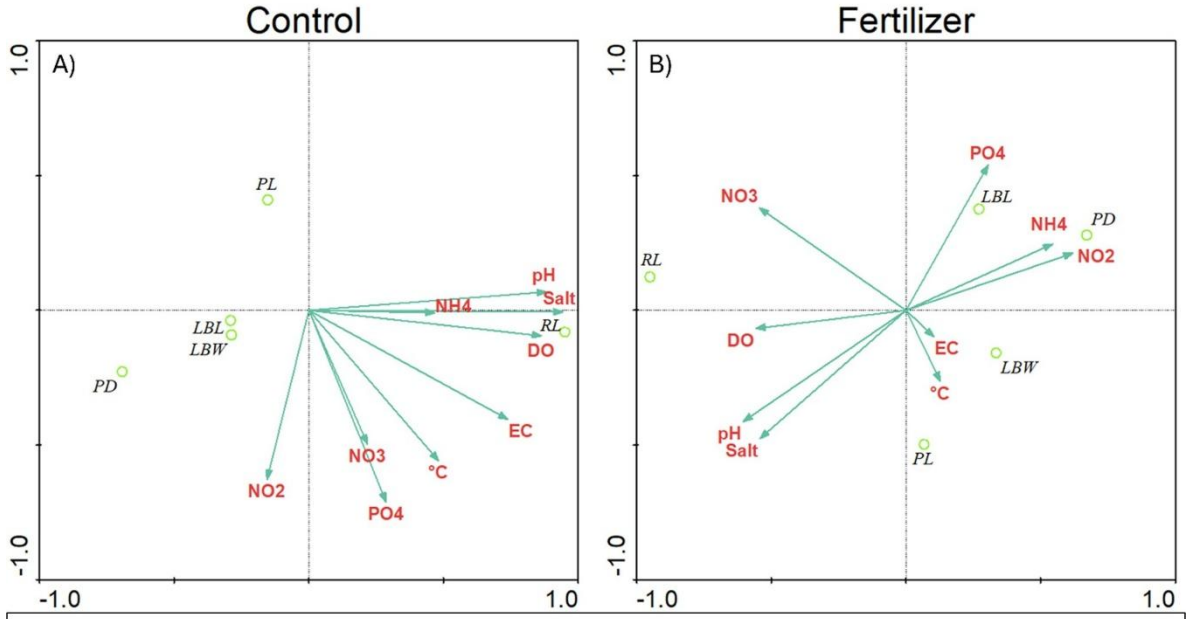


Figure 4: Relationship between physicochemical parameters and morphometric variables in both treatments: (A) Control, (B) Fertilizer. Physicochemical parameters: pH; DO (dissolved oxygen); Salt (salinity); EC (electrical conductivity); °C (temperature in °C); NO_2^-; NO_3^-; NH_4^+; PO_4^+. Morphometric variables: PD (petiole diameter); PL (petiole length); RL (root

3.4. Morphometric relationships

Finally, a difference can be observed (Fig. 5) between the biomasses of each treatment where the highest relative growth rate at the end of the bioassay was the control treatment, these rates were: 2.96 g/day for the control treatment and 1.89 g/day for the nutrient-enriched treatment, in turn, the root:shoot ratio was also higher in the control treatment than in the nutrient-enriched treatment (0.2692 g and 0.2260 g respectively).

3.5. Relative growth rate

Finally, it can be observed (Fig. 5) that greater biomass production occurred at the end of the bioassay in the control treatment. This was also corroborated by the relative growth rate calculations at the end of the experiment, which were 2.96 g/day for the control treatment and 1.89 g/day for the fertilizer treatment.

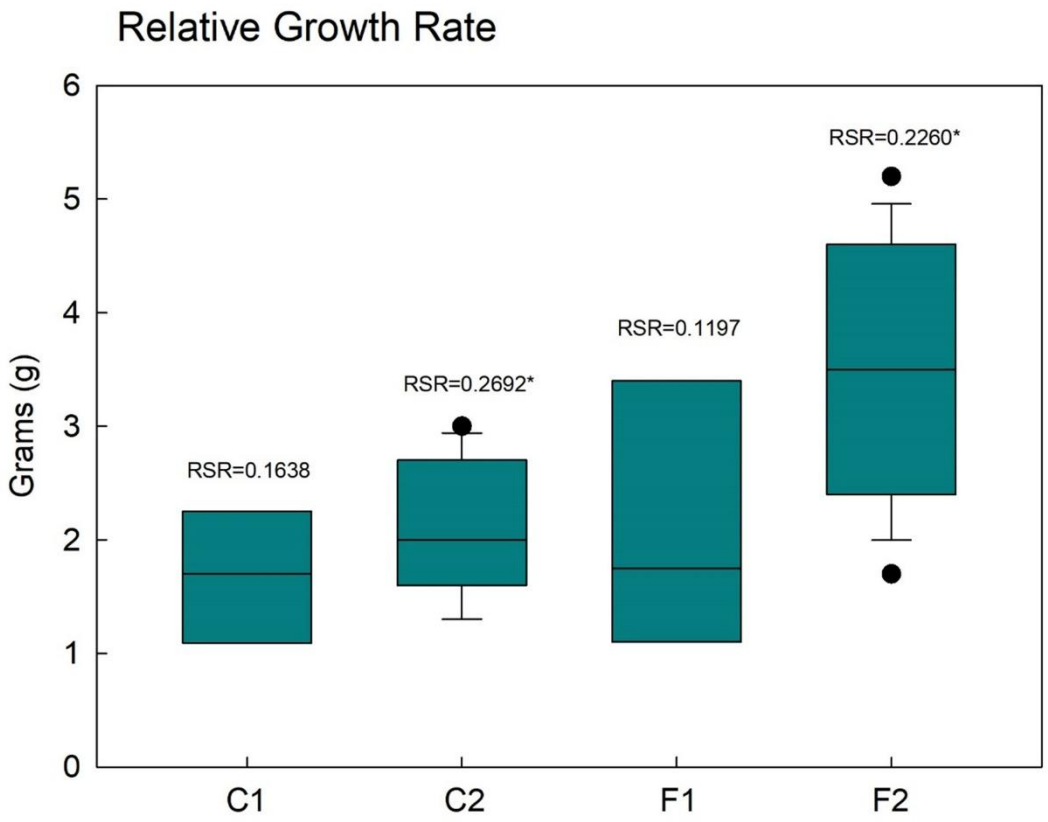


Figure 5: Relative growth rates at the beginning and end of the bioassay. C1) Control treatment at the beginning of the bioassay; C2) Control treatment at the end of the bioassay; F1) Nutrient-enriched treatment at the beginning of the bioassay; F2) Fertilizer-enriched treatment at the end of the bioassay; RSR) Root-

177

178 **4. Discussion**

179 *E. crassipes* is a species commonly used for phytoremediation purposes because
180 it is a hyperaccumulator plant of contaminants, such as heavy metals, emerging
181 contaminants, or nutrients, the latter are retained in the root zone, in turn giving it
182 high growth rates due to the utilization of these [25, 47]. According to the results of
183 the physicochemical parameter monitoring (Fig. 2 and Fig. 3), visual differences
184 between treatments were observed. This was corroborated by a two-factor ANOVA
185 ($p<0.001$) for DO, EC, salinity, and pH, which was subsequently confirmed with a

Holm-Sidak post hoc test ($p<0.05$). However, there were no differences in depth, indicating that the physicochemical conditions remained homogeneous across both treatments.

Among the effects of *E. crassipes* reported in bodies of water is the decrease in pH of the medium, which is greater compared to other macrophytes [26, 11]. Furthermore, the presence of phosphate and nitrogen functional groups, in combination with root exudates, such as various organic acids from this species, buffers against environmental acidification [40]. However, this bioassay shows an increase in pH in both treatments, with a statistically significant difference ($p<0.005$), corroborated by a confirmatory test (Holm-Sidak, $p<0.05$). This could be due to evaporation from the mesocosm medium. Similarly, pH values between 5 and 8 are optimal for the growth of this species; however, higher pH values may favor the removal of some contaminants that act as nutrients. [56].

DO showed differences among treatments, with a significant difference between treatments according to ANOVA ($p<0.001$), but not between depths. This was confirmed with a post-hoc test (Holm-Sidak $p<0.05$). The decrease in DO reported in the literature is due to its consumption by aquatic macrophytes [26]. Conversely, Sricoth et al. [54] reported an increase in DO concentration in a period of time similar to that of this bioassay, since maximum DO levels of 6 mg/l were obtained in the presence of a combination of 2 macrophyte species, while in this assay, maximum levels of 3.5 mg/l were reported using only *E. crassipes*. This indicates that oxygenation processes of the medium are carried out through the roots of this species, which is favored by the presence of nutrients.

The high levels of salinity and EC shown in the nutrient-enriched treatment are correlated with each other, which means that the trends in terms of their concentration will be the same [5, 12, 23, 32, 66]; the high concentrations may be a consequence of the interaction of physicochemical variables, such as high temperatures, low concentrations of DO, high levels of total dissolved solids, as well as nutrients in solution such as P and N or as a consequence of physical phenomena such as evaporation of water in closed media [17, 44].

On the other hand, there is a difference between the nutrient removal of the treatments according to the two-way ANOVA ($p<0.001$) with a Holm-Sidak post-hoc test (0.05) for the three nitrogen forms; however, this difference does not exist between depths, which contrasts with Gaballah et al. [18] where greater removal was achieved at the greatest depth (0.35 m). In turn, the surface concentration of NO_3^- will be due to the area with the greatest influence of the macrophyte; this is important because this nitrogen form is most readily assimilated [3, 43, 57]. However, this removal was influenced by the surface vegetation cover (90% cover) managed in their study. In turn, Adelodun et al. [1, 2] reported that the greatest removal (45%) occurred after 3 weeks under shallow conditions (final volume of 2 L), indicating that total depth is a variable that influences nutrient removal.

The greatest removal of PO_4^+ with a significant difference (ANOVA $p<0.001$, Holm-Sidak $p<0.05$) was observed at depth II with 35.95% by day 14, which contrasts with De Lima et al. [13], where 10% was removed. Conversely, Gaballa et al. [18] reported 84.2% phosphate removal in seven days. This may be due to greater contact with the total volume of the medium in the rhizosphere of their mesocosms

and to the extent of vegetation cover in their experiment, which could also have influenced the removal efficiency. However, the records from this bioassay show that the presence of *E. crassipes* prevents phosphorus precipitation, maintaining a homogeneous concentration in the mesocosm water column.

Several factors can enhance bioremediation efficiency by reducing nutrient concentrations. These include greater mixing combined with shallower depth, which implies greater removal efficiency [44]. Similarly, the hydraulic retention time and the density of vegetation cover around *E. crassipes* roots are also important, since the greatest nutrient removal occurs with longer contact time in the rhizosphere [37, 56]. Other factors that can affect the efficiency of nutrient removal and distribution in the water column are the plant composition [54], the synergistic effect of high temperatures, anoxic conditions, and slightly alkaline pH together with the metabolic processes of microorganisms in the environment [65, 13, 57], as well as the presence of dissolved metal ions with which phosphorus and nitrogen can bind [17].

On the other hand, environmental adversities for *E. crassipes* can promote trade-offs to buffer the effects of environmental stress, This is why high concentrations of nutrients turn out to be toxic to the species, and one way to overcome this is: the reallocation of resources from biomass production (production and growth of organs) to the production of clonal individuals, even if this implies the death of the parental individual [53].

Finally, a significant difference was observed according to the Kruskal-Wallis test of $p<0.001$ (Tukey $p<0.05$) in the biomass produced by the individuals of the

treatment enriched with fertilizer (Fig. 5). This indicates the distribution of plant biomass, so a low value indicates a relatively equitable distribution of nutrients, while a high value of this metric indicates nutrient deficiencies and/or low biomass production. Thus, the higher relative growth rate of the control treatment may be due to the reallocation of resources due to nutrient deficiencies reflected in the root:shoot ratio, where, in turn, there was a greater production of stolons, which implies a greater number of individuals (variable not quantified), as well as the senescence rate of the parent organism [49].

5. Conclusion

E. crassipes can improve the physicochemical conditions of the environment, specifically by increasing its oxygenation. In turn, its presence can significantly decrease high nitrogen concentrations (mainly in the form of nitrate), while preventing phosphorus precipitation in shallow environments, thereby facilitating its subsequent removal from the water column by *E. crassipes*.

The physiological capabilities of this species make it a biological model for phytoremediation, as it not only improves the physicochemical conditions of the environment but also withstands stressful conditions, such as high nutrient concentrations. Its clonal reproduction capacity, presented as a trade-off, not only favors the species' survival but also increases the effective area for removal, functioning as a bioaugmentation strategy for bioremediation.

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Data availability

All data generated by this investigation and complementary material are not published due to the data volume but are available upon request from the author.

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