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## Abstract

Aquatic macrophytes act as water filters that purify water bodies by the accumulation of metals in their plant tissue. This study compares the potential of two floating macrophytes species: *Pistia stratiotes* and *Eichhornia crassipes* collected from Lake Cidra, Puerto Rico to bio-accumulate heavy metals. The focus of the study was to evaluate ability of these two species to bio-accumulate five nutrients: nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg) and sodium (Na), potassium (K) and 4 heavy metals: copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). This study reveals a difference in the capacity of these two species to bio-accumulate the above-mentioned nutrients and heavy metals. The heavy metals concentration found in these plants tissues is higher when compared to that of the five nutrients analyzed, whereas, the concentration of heavy metals present in the water samples was low. In contrast, the concentration of nutrients in the water samples was high as compared to that present in the plants. In summary, we found *P. stratiotes* to have a higher bio-accumulation capacity for the sampled elements than *E. crassipes*, especially to heavy metals.

Keywords: Pistia stratiotes; Eichhornia crassipes; Heavy Metals; Phytoremediation; Puerto Rico; Macrophyte

### Introduction

Cidra Lake is a reservoir built in 1946 for water storage in the central region of Puerto Rico. The Island lacks natural lakes; therefore, 36 reservoirs were built to provide fresh water to the population. Since its construction, various aquatic weeds, specifically water hyacinth *(Eichhornia crassipes)* and aquatic lettuce (*Pistia stratiotes*) have invaded the reservoir through the years. These macrophyte species, exotic to Puerto Rico, can dominate an ecosystem. Their adaptive skills are evidenced by their impressive growth rate and ability to displace native species. Without their natural predators, these macrophytes directly affect the ecosystem's biodiversity and ecological balance [1], increasing biotic homogenization in the ecosystem. This process is the increased similarity of biota as a result of introductions of non-native species [2]. Once established, its control and eradication imply a significant impact to the local economy [3]. Therefore, macrophytes represent ecological, social, and economic problems such as a decrease in biodiversity, eutrophication, plagues and adverse effects to agriculture and aquaculture. In addition, they can affect recreational activities and eventually affect water quality [4,5]. In general, macrophytes limit the uses of the reservoir [6-10] and the presence of only one species can alter an entire ecosystem [11]. However, their rapid growth can be beneficial while using the organisms in the phytoremediation of nutrient enriched ecosystems.

Thus, the presence of aquatic weeds in reservoirs, specifically *Pistia stratiotes* and *Eichhornia crassipes* are an indication of possible contamination and eutrophication, two environmental issues that can affect surrounding communities.

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### **Purpose of the Study**

The main purpose of this study is to evaluate ability of these two species to bio-accumulate five nutrients: nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg) and sodium (Na), potassium (K) and four heavy metals: copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn).

## **Materials and Methods**

#### **Field sampling**

Sampling sites were selected after site visits and identification of the *Eichhornia crassipes* and *Pistia stratiotes* in the field. This to compare the bioaccumulation capacity of the two species, in the environment of Lake Cidra in Puerto Rico. We sampled the area of the Sabana bridge with coordinates: 18°10'31"N, 66°9'12"W in the entrance of the Treasure Valley community in the Bayamón neighborhood in Cidra (Figure 1). Five samples of each species were collected. Each sample of *P. stratiotes* was composed of five plants including leaves, stalks, and roots. On the other hand, each sample of *E. crassipes* was composed of three plants including leaves, stalks and roots. The samples were collected and rinsed with distilled water to eliminate most of the mud and insects and stored in 16 oz. plastic bags. Water samples were collected in each area where the vegetative samples were collected. The water samples were placed in 16 oz plastic bottles. These were stored immediately in a cooler to ensure that the physical properties of the water samples were maintained. All the samples were transported to the laboratory for analysis.



Figure 1: Location of study site.

#### **Data analysis**

Laboratory analyses were conducted to determine the concentration the nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) and heavy metals: copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). The chemical analysis of nitrogen in the vegetative tissue was performed by the Kjeldahl or EPA 351.1 method. In the case of phosphorus, L-Ascorbic Acid+NH<sub>4</sub>Mo+Antimony Potassium Tartrate or EPA 365.1 method was used. Potassium, Calcium, Magnesium, Manganese, Copper, Nickel

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and Iron were extracted and analyzed by dry ash or EPA 600 method. For the water analysis, Atomic absorption spectroscopy or EPA 7000 B Method was used for all nutrients and heavy metals analyzed: P, K, Ca, Mg, Na, Cu, Mn, and Zn. All the results were read by UV visible spectrophotometer. Results were compared and analyzed using statistical tests to establish a difference between species for the analyzed elements. With the results, average, standard deviation, and the bioaccumulation factor (BAF) were calculated. BAF is the proportion of the concentration of the element analyzed in the plant and in the water [12]. The BAF formula was used as described by Zewge., *et al.* [13] and Kumar, *et al.* [14] in their investigations:

## $BAF = C_B / C_W$

The BAF was only calculated for the analyzed nutrients since the heavy metal concentration in water was too small to be detected by the laboratory analysis. Hence, BAF could not be calculated for the analyzed heavy metals.

The Shapiro-Wilks test was used to determine if there was a normal distribution for the analyzed elements in the samples. The t test was used to compare the bioaccumulation results between the two species and determine if there was a significant difference between them.

## **Results and Discussion**

We compared the results obtained for the elements analyzed for each species. After establishing a normal distribution among the results using the Shapiro-Wilks test, we used the *t* test to establish a difference in the bio-accumulation capacity between the two species. When *t* test was calculated, we obtained a significant difference between the species for nitrogen, phosphorus and magnesium, a very significant difference for potassium and iron and an extremely significant difference for calcium and manganese. Although *P. stratiotes* bioaccumulated more zinc and copper than *E. crassipes*, no significant difference was found. We calculated the standard deviation for each of the analyzed elements and obtained very low results for the analyzed nutrients N, P, K, Ca, Na and Mg (SD < 0). These results showed a very slight difference between the samples taken. However, when we calculated the standard deviation for the analyzed heavy metals Cu, Fe, Mn and Zn, much higher results were observed, specifically for Ca, Na and Mg. This means that the results between the heavy metals concentration varies much more for the samples taken each of the species. These differences between species could be sexually related since both species reproduce both, asexually and sexually [15]. These differences could be justified by different morphotypes of the same species or could be age related.

The formula  $BAF = C_B/C_W$  was used to calculate the bioaccumulation value of the analyzed nutrients. The BAF could not be calculated for the heavy metals since there were no perceptible concentration for the heavy metals analyzed in the water samples; thus, confirming the superior bio-accumulating capacity for heavy metals of both species. The BAF values for the analyzed nutrients show that the bio-accumulation capacity for the analyzed nutrients is low, although their concentration in water was significant.

From the elements analyzed, we found that *Pistia stratiotes* have a better bio-accumulation capacity than *Eichhornia crassipes*. Student's t-test were conducted and were considered significant if calculated p-values were lower than 0.05 (Table 1).

	N	Р	К	Са	Mg	Cu	Fe	Mn	Zn
t	2.79	2.32	4.16	5.46	2.37	1.52	4.06	9.79	1.50
р	0.024	0.049	0.003	0.0006	0.046	0.17	0.010	0.006	0.11

Table 1: Summary for t test results for the analyzed elements.

*t* = Statistical value for *t* test.

p = Probability value. 0.05 was used.

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This is the case for nitrogen, phosphorus, calcium, magnesium, copper, iron, manganese, and zinc (Figure 2). In the case of zinc and copper, the bioaccumulation difference was not significant (Figure 2C and 2D). Only in the case of potassium, *E. crassipes* obtained a bigger concentration than *P. stratiotes* and was confirmed when the *t* test was applied, and a very significant difference was obtained. We analyzed the results and found that *P. stratiotes* exceed *E. crassipes* by 24% in the bioaccumulation capacity for nitrogen and 60% in the case of phosphorus. As per Lu., *et al.* [16], *P. stratiotes* demonstrates a great potential for removing N and P, especially in eutrophic ecosystems. *P. stratiotes* exceeds by 43% *E. crassipes* in the case of calcium and in the case of magnesium by 13%. Finally, *E. crassipes* exceeds the bioaccumulation capacity of *P. stratiotes* by 56% in the case of potassium. On one the hand, for the analyzed heavy metals, *P. stratiotes* exceeds by 35% *E. crassipes* in the bioaccumulation capacity for zinc. In the case of copper, *P. stratiotes* exceeds by 59% *E. crassipes*. In the case of iron, *P. stratiotes* exceeds by 42% *E. crassipes* and in the case of copper, *P. stratiotes* exceeds by 19% *E. crassipes*. These results coincide with the results of Begum [17], Yasar, *et al.* [18] and Lu., *et al.* [19], which confirm the great bioaccumulation capacity for heavy metals of *P. stratiotes. E. crassipes* has also demonstrated an affinity to bioaccumulate heavy metals [20,21]. Both plants show the same bio concentration order for the analyzed heavy metals: Fe > Mn > Zn > C, which concur with Kumar, *et al.* [20] and Mishra, *et al.* [22] for *E. crassipes.* We found a difference in the bioaccumulation capacity for the analyzed nutrients. In the case of *P. stratiotes* we observed the following bioaccumulation order: Ca > N > K > Mg > P and in the case of *E. crassipes* as follows: K > Ca > N > Mg > P.



Figure 2: Concentration of Nutrients on A-Pistia stratiotes, B-Eichhornia crassipes and Heavy metals concentration on C-Pistia stratiotes and D- Eichhornia crassipes.

#### Conclusion

In this study a comparison was made between the concentration of nutrients and heavy metals in water samples and vegetative material of two macrophytes, *Pistia stratiotes* and *Eichhornia crassipes*. The results presented in this study show that the two macrophytes studied display a high concentration of heavy metals in the vegetative tissue. A high concentration of 2418 ppm in the case of iron, 1961 ppm in the case de manganese, 275.40 in the case of zinc and 23 ppm in the case of copper in the vegetative tissue of the species *Pistia stratiotes* (Figure 2C and 2D). Likewise, we observed an elevated concentration of 1765 ppm in the case of iron, 927 ppm in the case de manganese, 268.50 ppm in the case of zinc and 26.10 ppm in the case of copper in the vegetative tissue of the species *Eichhornia crassipes* 

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(Figure 2C and 2D). These results are consistent with a high bio-accumulating capacity for heavy metals in both species and validates the possible use of these plants in phyto-remediation. Their capacity for concentrating heavy metals may turn them into possible contamination focal points when the plants die and start decomposing, since it increases the concentration of these heavy metals in the sediments of the aquatic ecosystem [23]. This should be taken in consideration when using the species for phyto-remediation.

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