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PAPER

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Temporal and physiological influence of the absorption of nutrients and toxic elements by *Eichhornia crassipes*

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Aquatic macrophytes are a very important subject of study due to their capacity to restore polluted aquatic environments as they need high nutrient concentrations to develop. The present study aims to determine their temporal and physiological influence on the amount of total nitrogen, gross protein, P, Cu, Ni, Co, Cd, Pb and Cr absorbed by *Eichhornia crassipes* (water hyacinth) in the River Apodi/Mossoró, RN, Brazil, identifying viable possibilities for the use of cultivated biomass. Results obtained from the parameters analyzed show that these substances are impacted by the temporality and physiology of *Eichhornia crassipes*. Leaves showed higher crude protein and macronutrients, while the content of micronutrients and toxic elements was higher in roots. It could, therefore, be utilized to improve water quality in the River Apodi/Mossoró.

1 Introduction

The Hydrographic Basin of the River Apodi/Mossoró in the state of Rio Grande do Norte is the most important surface water resource in the eastern region of Northeast Brazil (Martins *et al.*) and extends from its source in the city of Luis Gomes to its mouth between the cities of Grossos and Areia Branca. It is 210 km long, occupies approximately 14.276 km^2 (26.8% of the state) and has a flow rate of around 360 million m^3 per year.[1] The river plays a vital economical role in the area. This is apparent from the agricultural activity and micro-companies on its margins, as well as fishing and other economic enterprises. All of these activities are common at locations where untreated effluents are generated.[1]

The estimated worldwide expenditure on environmental cleaning is approximately 25 to 30 billion dollars a year.[2] However, treating polluted aquatic environments using aquatic macrophytes is of low-cost and the biomass produced can be recycled. Depending on its quality, it can be used for energy production (biogas or direct burning), producing ethanol and low-cost bricks, as well as in handicrafts, the production of protein concentrate for human consumption, protein extraction for animal feed and paper manufacture.[3–7]

In order to counterbalance problems generated by human activity, more efficient methods of restoring aquatic environments are being sought. Macrophytes are studied for their potential to filter polluted rivers and lakes of industrial effluents and other waste, since they need high nutrient concentrations to develop. However, using these plants in environments with high eutrophication levels can interfere with the uses of the rivers, lakes and reservoirs in many ways. These include hampering navigation and water extraction, jeopardizing electrical energy production and leisure activities, obstructing water flow in

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Environmental impact

In places with high pollution, the aquatic macrophytes are developed with tremendous speed, causing the total eutrophication of these environments. Several studies have been conducted to monitor the entire course of the River Apodi/Mossoró. However, there are no studies dealing with issues related to decontamination of its waters. Thus, this paper proposes a way to improve the quality of its waters, and hence the quality of life. To make this possible it is necessary to know the influence of factors such as temporal and physiological processes in the remediation of these environments, little studied in natural environments, so you can get through the process of phytoremediation, the best possible results.

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irrigation canals and contributing to the reduction of dissolved oxygen in the water.[8–11] The use of macrophytes for whatever means depends on determining several physicochemical properties typical to the intended application and the accumulated pollution content, as well as compliance with legislation. The application of macrophytes for nutrient removal in temporary rivers that appear during the dry season and where pollution content is seasonal has not yet been fully studied. Confirming their efficiency would be an important economical innovation for the region.

Worldwide use of aquatic macrophytes to filter aquatic environments has increased substantially. This is apparent from the numerous studies found in the literature. In Brazil, studying these plants as potential phytoremediators has intensified significantly in recent years. This is primarily in view of their low cost and broad applicability in diverse substance groups. The aquatic macrophytes of the species *Eichhornia crassipes* (water hyacinth) are floating aquatic plants from the *Pontederiaceae* family. They are very common in tropical areas and reproduce mainly through stolons (horizontally growing shoots). In Brazil, the species occurs in both natural aquatic ecosystems and in aquatic environments affected by anthropic activities. In the latter, the plants have elevated growth rates and produce large quantities of biomass.[12,13]

The present study aims to determine the temporal and physiological influence of the amount of total nitrogen, gross protein, total phosphorous, copper, nickel, cobalt, lead and total chromium absorbed by these macrophytes (*Eichhornia crassipes*) in the River Apodi/Mossoró, to identify possible uses for the biomass produced.

2 Methodology

The experiment was divided into 4 harvests carried out at a point in the section of the River Apodi/Mossoró (main river bed) flowing through the town of Mossoró, Brazil. The site was strategically chosen based on a variety of factors, including water volume, physical structure, anthropogenic factors, location and others (Fig. 1).

The four harvests were performed in August, October and December 2008 and February 2009, wherein August 2008 is characterized by the end of the rainy season and early dry season, and February 2009, end of dry season and early rainy season (Fig. 2 and 3).

Fig. 1 Harvest site in the River Apodi/Mossoró, Brazil.

Analyses were carried out in accordance with the methodology described in the Manual for the chemical analysis of soil, plants and fertilizers of Brazilian Agricultural Research Firm,[14] as well as the Analytical Norms of the Adolfo Lutz Institute.[15] These are briefly described below.

In all the harvests, 32 plants were randomly collected, stored in plastic bags containing a small amount of water from the actual sites and sent for prior cleaning and treatment. Plants were separated into leaves (blade and petiole) and roots, washed with tap water and rinsed abundantly with distilled water. They were then dried with paper towel and underwent quantitative determination. The leaf area was determined with a LI-COR 3100 leaf area meter, previously calibrated in accordance with its manual. It is worth noting that as only the blade area is considered the leaf area and was determined by randomly separating 32 plants in 8 repetitions of 4 plants each.

Humidity content was measured in the blade and petiole. These were weighed *in natura* following moisture loss (dehydration), which occurred after seven days of heating at 60 °C in a forced air oven. Dry leaf mass was established by weighing the blade after dehydration under the same conditions as those used to determine moisture. Once dry, the leaves (blade and petiole) and roots were ground separately in a blender with stainless steel blades and stored for later analysis in previously washed and dried plastic flasks. Total nitrogen content was calculated in triplicate, in accordance with the Kjeldahl method and gross protein content by multiplying the nitrogen values by 6.25. For the determination levels of total phosphorous and metallic cations in the plant tissue were held, in triplicate, the dry digestion process in a electric furnace at 500 °C for 3 hours, followed by the dissolution of the ash with hydrochloric acid solution 1.0 mol L^{-1}.[14] Levels of total phosphorus were determined by molecular absorption spectroscopy (MAS), and cobalt, copper, nickel, total chromium, cadmium and lead were determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Data obtained underwent statistical treatment *via* variance analysis in randomly assigned subdivided plots. Plant matter (leaves and roots) were determined as plots and the different time periods (August, October and December 2008 and February 2009) as subplots. Mean values were compared using Tukey's test with a 5% probability level and the ESTAT V. 2.0 Statistical Analysis System.

3 Results and discussion

Determining the leaf area was fundamental to interpret the results. This is because, in accordance with the size or stage of plant development, changes in this variable may reflect on its capacity to absorb nutrients and other substances (pollutants or non-pollutants) present at the site studied.

Leaf area values at the location studied increased with growth time and only became constant in the final harvest (Table 1). This behavior was previously observed in *Eichhornia crassipes* by Henry-Silva and Camargo[18] in cultivated plants grown in experimental units, where continuous water flow was provided by freshwater shrimp farms.

Moisture data did not undergo statistical analysis to differentiate the four time periods. However, the mean value of this

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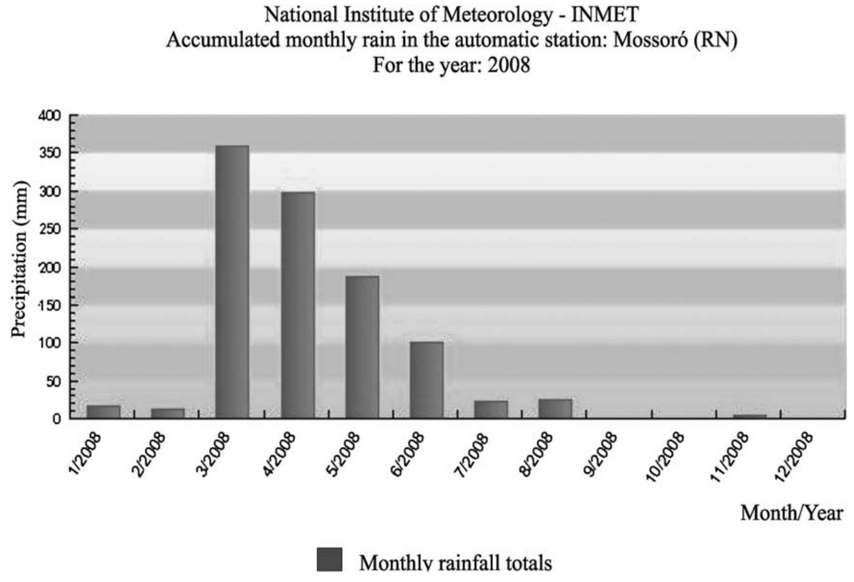

Fig. 2 Climatological data from the city of Mossoró, RN, Brazil, in 2008. Source: INMEP.

property is relevant to the objective of this study and is therefore presented in Table 1. Mean moisture content recorded (91.63%) was in accordance with the literature, and only slightly higher than the value between 80 and 90% found in most plants, terrestrial or aquatics. This varies according to the environment.[16] Ascertaining this parameter is significant, especially when the material is proposed for use in a study. This is because mechanical or physical processes may be necessary to remove moisture from the material, depending on its moisture content. This increases its production costs and is an economically important factor in its viability.

In relation to dry leaf mass, the effects of interaction between harvest periods were not significant at a 5% probability level. However, the values suggest behavior that increases with plant growth (Table 1). This occurs because all values are small and nearby at different times of harvest.

Total nitrogen values in *Eichhornia crassipes* leaves decrease from the first to the fourth harvest (Fig. 4 and Table 1), which is probably due to three factors:

(a) The physiology of the plant, since nutrient absorption levels tend to fall as the plant matures.[17]

(b) Competition between macrophytes: due to their large numbers, there is a lack of surrounding space and the plants generally grow vertically, as confirmed in previous studies.[18] Consequently, the petiole is substantially more developed than the blade. Since the protein content is significantly lower in the petiole, a dilution effect occurs.[19]

(c) Nitrogen levels in the water, given that the nutrients absorbed by the macrophytes originate in the environment where they were cultivated.

The same is true for gross protein levels, which are directly proportional to total nitrogen values (Fig. 4 and Table 1).

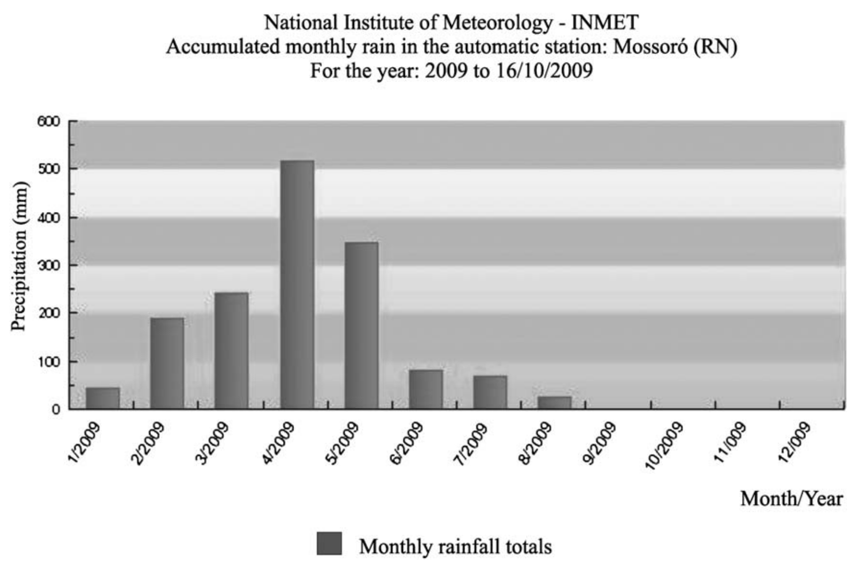

Fig. 3 Climatological data from the city of Mossoró, RN, Brazil, in 2009. Source: INMEP.

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Table 1 Mean ± standard deviation of results obtained at different times and on different parts of the plants[a]

Parameters	Plant parts	Collection periods August/08	October/08	December/08	February/09	C.V.[b]	C.V.[c]
Leaf area (cm²)	Leaf	23.17 ± 2.79 *C*	67.21 ± 6.01 *B*	88.59 ± 9.87 *A*	100.29 ± 14.00 *A*	—	16.02
Moisture (%)	Leaf	91.62 ± 5.79	92.06 ± 5.82	91.68 ± 7.06	91.17 ± 8.62	—	—
Leaf dry mass (g)	Leaf	0.0913 ± 0.0187 *ns*	0.2745 ± 0.0221 *ns*	0.4448 ± 0.0667 *ns*	0.5034 ± 0.1099 *ns*	—	—
Total nitrogen (g/100 g)	Leaf	4.4088 ± 0.0747 *aA*	3.1649 ± 0.0117 *aB*	2.1241 ± 0.0348 *aC*	1.5256 ± 0.0177 *aD*	1.20	1.67
	Root	2.0306 ± 0.0124 *bA*	1.8522 ± 0.0153 *bB*	1.9761 ± 0.0372 *bA*	1.4595 ± 0.0238 *bC*		
Gross protein (g/100 g)	Leaf	27.5549 ± 0.4666 *aA*	19.7805 ± 0.0730 *aB*	13.2755 ± 0.2176 *aC*	9.5351 ± 0.1106 *aD*	1.11	1.76
	Root	12.6911 ± 0.0777 *bA*	11.5761 ± 0.0953 *bB*	12.3507 ± 0.2328 *bA*	9.1218 ± 0.1488 *bC*		
Total phosphorous (g/100 g)	Leaf	0.4483 ± 0.0029 *aC*	0.6416 ± 0.0018 *aA*	0.5654 ± 0.0040 *aB*	0.4234 ± 0.0044 *aD*	0.78	0.66
	Root	0.2523 ± 0.0007 *bC*	0.3000 ± 0.0006 *bB*	0.3590 ± 0.0082 *bA*	0.2349 ± 0.0028 *bD*		
Copper (mg/100 g)	Leaf	0.8747 ± 0.0576 *aB*	1.5779 ± 0.0612 *bA*	1.5723 ± 0.0556 *aA*	0.3029 ± 0.0125 *aC*	9.60	4.98
	Root	0.7775 ± 0.0217 *aC*	2.8901 ± 0.1821 *aA*	1.4670 ± 0.0113 *aB*	0.4271 ± 0.0734 *aD*		
Nickel (mg/100 g)	Leaf	0.1180 ± 0.0016 *bA*	0.0969 ± 0.0023 *bA*	<LOD	<LOD	2.20	2.58
	Root	0.4851 ± 0.0121 *aA*	0.4057 ± 0.0120 *aB*	0.3083 ± 0.0109 *C*	<LOD		
Cobalt (mg/100 g)	Leaf	<LOD	<LOD	<LOD	<LOD	—	5.10
	Roof	0.3721 ± 0.0112 *A*	0.2375 ± 0.0012 *C*	<LOD	0.2706 ± 0.0096 *B*		
Chrome (mg/100 g)	Leaf	<LOD	<LOD	<LOD	<LOD	—	6.18
	Root	1.0699 ± 0.0297 *A*	0.5935 ± 0.0247 *B*	0.1969 ± 0.0004 *C*	<LOD		
Cadmium (mg/100 g)	Leaf	<LOD	<LOD	<LOD	<LOD	—	—
	Root	<LOD	<LOD	<LOD	<LOD		
Lead (mg/100 g)	Leaf	<LOD	<LOD	<LOD	<LOD	—	—
	Root	<LOD	<LOD	<LOD	<LOD		

[a] Means followed by lower-case letters in the columns and upper-case letters in the rows for each individual parameter do not differ significantly using Tukey's test with a 5% probability level. [b] Coefficient of variation for the plots. [c] Coefficient of variation for the subplots. ns = not significant. <LOD = lower than the limit of detection for the equipment used.

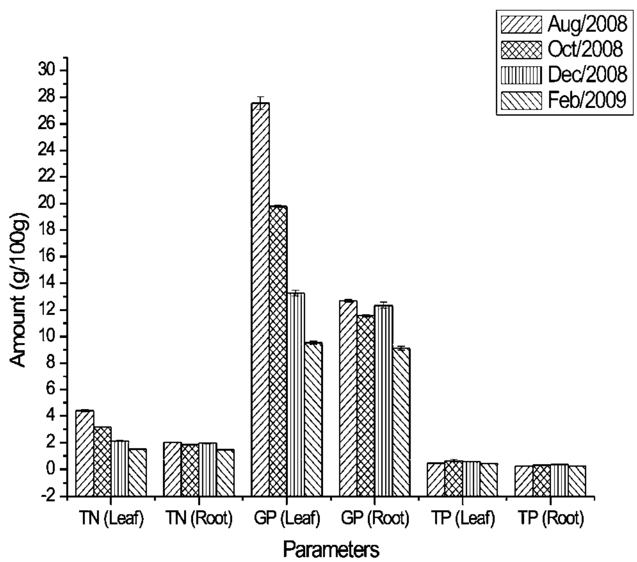

Fig. 4 Content variation of total nitrogen, gross protein, and total phosphorous (g/100 g dry matter) at different times and in different parts of the plants. TN = total nitrogen; GP = gross protein; and TP = total phosphorous.

This behavior was also observed in the roots, although an exception was recorded in December 2008. A significant increase was found in the amount of total nitrogen when compared with the previous harvest. This is probably due to higher nitrogen concentrations in the environment caused by the lower water volume during this period.

When comparing total nitrogen and gross protein values found in the leaves with those in the roots, the latter present significantly reduced levels. Given that higher quantities are essential for plant development, it is a predominant factor in facilitating the transport of nitrogen from the roots to the leaves, which increases concentrations in these areas.

According to these observations, it is necessary to apply a rotating system of harvesting macrophytes. This will result in greater control over the age of plants, favoring the use of young macrophytes, which have higher gross protein content.

Henry-Silva and Camargo carried out studies using three different species of floating aquatic macrophytes to treat aquaculture effluents. Protein values of approximately 7.2% were recorded for *Eichhornia crassipes*, 8.8% for *Pistia stratiotes* and 8.7% for *Salvinia molesta*. All these results are lower than those obtained in the present study using *Eichhornia crassipes* present in the River Apodi/Mossoró. Despite variations in absorption capacities between species, the availability of nitrogen in the environment for plant absorption is also a limiting factor. Non-distinction between leaves and roots may also have influenced the protein values obtained in the present study. Joint analysis of both parts causes a dilution effect, which is not previously mentioned by the authors.[20]

According to data for total phosphorous (Fig. 4 and Table 1) and copper (Fig. 5 and Table 1), these concentrations rose until the second or third harvest, followed by a substantial decline. This behavior can be explained by the physiological changes in plant development and bioavailability of these nutrients in the water. Between the first and the third harvest the plants were in a development stage, which is apparent from their increase in size. During this phase, the plants have a high ambient nutrient absorption capacity. As they mature, absorption capacity decreases and nutrient levels in the plant matter fall (fourth harvest).[19]

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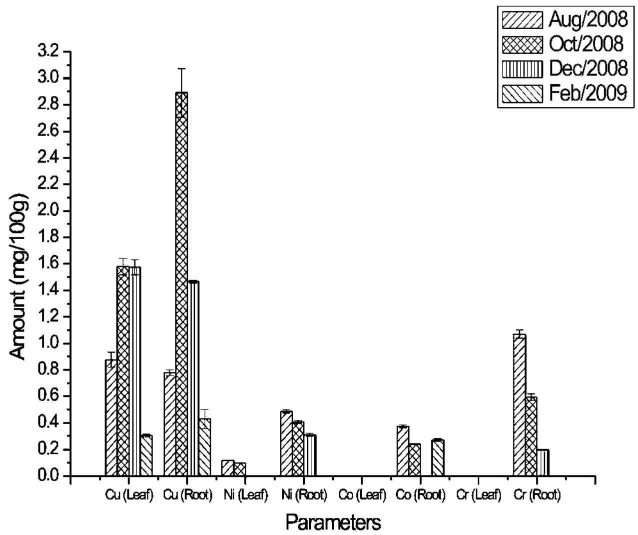

Fig. 5 Content variation of copper, nickel, cobalt and total chromium (mg/100 g dry matter) at different times and in different parts of the plants.

Comparison of total phosphorous values in different plant parts revealed the same behavior for total nitrogen and gross protein. The opposite was true for copper, which is probably due to the high copper concentrations in the river water. Given that the plants require only a small amount for their development (micronutrient), it tends to be absorbed as a function of the bioavailable amount and accumulate in the roots.

Henry-Silva and Camargo also obtained a total phosphorous content of 0.24%, which is very close to the result recorded in the present study.[20]

In relation to copper, studies have shown that *Eichhornia crassipes* is highly efficient at removing this nutrient from solutions with efficiencies of removal varying between 86 and 95%.[21] These data show that using this kind of macrophytes to recover contaminated environment for copper is a viable alternative.

Nickel content was significantly lowered with plant growth (Fig. 5 and Table 1) for the same reasons as previously cited for other parameters. Copper behaved in the same way when comparing the parts of *Eichhornia crassipes* analyzed. That is, higher concentrations were recorded in macrophyte roots.

The nickel content in this species was also ascertained by Martins *et al.*[22] These were recorded at 88.09 mg kg^{-1} and therefore substantially higher than those found in macrophytes in the River Apodi/Mossoró.

Considering leaf data presented for cobalt, cadmium, lead and total chromium, concentrations of these four elements were below the limit of detection. Cobalt and total chrome were found and quantified in the root and neither presented a behavior pattern with plant growth (Fig. 5 and Table 1).

These results indicate the possibility of leaf biomass use as animal feed. Roots should be removed and not put into the animal feed as their increased toxic element contents are undesirable for any organism. Roots could be used in brick manufacture, biomass conversion and metals recovery from the ashes.

Martins *et al.* determined cobalt, chromium and lead levels in several species of aquatic macrophyte. Contents recorded in plant total dry matter were 5.35 mg kg^{-1}, 76.19 mg kg^{-1}, and 0.00 mg kg^{-1}, respectively.[22] There is therefore a similarity between these values and the cobalt and lead values in roots (Table 1). However, the chromium content found by these authors was significantly higher than that obtained in roots from macrophytes in the River Apodi/Mossoró.

Gross protein values in *Eichhornia crassipes* samples collected from the River Apodi/Mossoró were compared with the amount of protein present in plants used for human consumption in accordance with the Brazilian table of food composition (TACO).[23] The aquatic plants were found to have levels substantially higher or similar to those considered rich in protein. They could therefore be a viable source for producing protein concentrate or derivatives used for animal or human consumption. In accordance with TACO, values recorded in some plant-based foods are: uncooked brown rice (7.3 g/100 g), wheat flour (9.8 g/100 g), raw crisp lettuce (1.3 g/100 g) and raw bean sprouts (4.2 g/100 g).

Phosphorous amounts in the roots were very similar to those found in some of the foods analyzed, while phosphorous and copper in the leaves and roots were higher. According to the TACO table, phosphorous and copper values in some foods are, respectively: 251.0 mg/100 g and 0.07 mg/100 g in uncooked brown rice, 115.0 mg/100 g and 0.15 mg/100 g in wheat flour, 26.0 mg/100 g and 0.03 mg/100 g in raw crisp lettuce and 75.0 mg/100 g and 0.17 g/100 g in raw bean sprouts.

Manufacturing simple organic fertilizers would be a more viable option, considering the amounts of nitrogen and phosphorous found in the plant. Another factor is its rapid growth, which produces 1 ton ha^{-1} day^{-1}, depending on the cultivation site.[24]

Normative Instruction N. 25 of July 23, 2009 establishes the norms and specifications, guarantees, tolerances, registration, packaging and labeling of simple, mixed and compound organic fertilizers and biofertilizers used in agriculture. It determines the maximum moisture content and minimum nitrogen value permitted in press cake and other simple organic fertilizers as 40% and 0.5%, respectively.[25]

A comparison of humidity values with those present in *Eichhornia crassipes* confirms that the water content in the plant tissue needs to be lowered. This can be accomplished by chemical or mechanical means. Nitrogen content in the macrophyte studied is significantly higher in both the leaves and roots during all the periods, which makes using the material in fertilizer production viable.

Another application of *Eichhornia crassipes* is in the manufacture of bricks used in civil construction. This form of using the biomass eliminates problems caused by the presence of toxic elements, since these are incorporated (stabilization) into the construction materials. It also allows for the building of low-cost housing.[3]

4 Conclusion

Levels of nutrients, gross protein and toxic elements in the plant tissue of aquatic macrophytes of the species *Eichhornia crassipes* were significantly influenced by temporal and physiological variability.

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On the whole, macronutrients and gross proteins are found in higher concentrations in the leaves, whereas raised levels of micronutrients and toxic elements were recorded in the roots. In view of their vital role in the development of the plants, these results were expected.

Total chromium is not beneficial to the development of *Eichhornia crassipes* and was therefore found in greater concentrations in the root. Cadmium and lead values in both parts of the plants were all below the limit of detection of the equipment used.

According to these observations, the biomass of *Eichhornia crassipes* in the River Apodi/Mossoró may have a variety of uses, provided there is a constant control of substance levels in the plant tissue. The aquatic macrophytes studied can be used in:

(a) Producing protein concentrate or derivatives for animal or human consumption, owing to the maximum amount of gross protein, phosphorous and copper found.

(b) Manufacturing simple organic fertilizers, in view of the total nitrogen content recorded.

(c) Manufacturing low-cost bricks.

As previously mentioned, there are several applications for the biomass produced. However, the uses described in the present study are the most appropriate for the reality and needs of the region.

The results obtained demonstrate that using the aquatic macrophyte species *Eichhornia crassipes* would improve water quality in the River Apodi/Mossoró and have a positive impact on the quality of life of the population.

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