

Temperature influences swine wastewater treatment by aquatic plants

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Edited by: Paulo Cesar Sentelhas

Received August 04, 2019

Accepted January 05, 2020

ABSTRACT: Studies show that aquatic plants are an efficient alternative to minimize the harmful effects of wastewater. Two species of aquatic macrophytes (*Pistia stratiotes* and *Eichhornia crassipes*) were chosen to evaluate the temperature effect on the performance of these macrophytes species in a post-treatment system of swine wastewater. The experiment was carried out in the municipality of Toledo, Paraná State, Brazil. For the experimental system, 24 concrete tanks with 80 L capacity were used, each measuring approximately 1.5 m in length, 0.25 m in width and 0.16 m deep. Wastewater characteristics and variables hydrogen potential (pH), electrical conductivity (EC), and dissolved oxygen (DO) were measured weekly. After, we analyzed the aquatic macrophytes and centesimal composition. The use of aquatic macrophytes was efficient for final swine wastewater polishing. Nitrogen removal showed higher efficiency in the summer period for both species studied. The abiotic variables (pH and EC) presented better results during the winter for both species evaluated. Air and wastewater low temperatures did not influence these variables. Dissolved oxygen presented better results in the summer period for *E. crassipes* species. There was a significant increase in N and P contents in the plant tissue. This fact proves that the use of aquatic macrophytes is an interesting alternative for swine wastewater treatment.

Keywords: *Pistia stratiotes*, *Eichhornia crassipes*, nitrogen, phosphorus, macrophytes

Introduction

In the last ten years, Brazil has increased its swine production by 3 million tons per year, reaching 3.75 million tons in 2017. Most production is consumed by the domestic market (81.5 %), and the states of Paraná, Santa Catarina, and Rio Grande do Sul account for 68.9 % of pig slaughters in 2017 (ABPA, 2018). However, this practice is considered by environmental agencies as a potential source of contamination and degradation of air, water resources, and soil (Rasheed et al., 2018). Studies point to aquatic plants as an efficient alternative to mitigate the harmful effects of wastewater (Gomes et al., 2016). In phytoremediation, plants absorb water and nutrients, as well as pollutants, through their roots, acting as a biological filter. In this sense, the capacity of these plants can be enhanced with the application of species better adapted to bioaccumulation of specific pollutants (Dudai et al., 2018; Gajić et al., 2018). However, to improve the efficiency of aquatic macrophytes as a filter for wastewater, it is necessary to understand how some environmental factors can alter this efficiency. Temperature is one of the limiting factors that influences the primary productivity of macrophytes (Kirk, 1994), since it controls the speed of chemical reactions (Guo et al., 2019). Nevertheless, its ideal temperature range tends to vary among species and case studies are needed to better understand these dynamics (Geneviève et al., 1997).

In our study, we chose two species of aquatic macrophytes (*Pistia stratiotes* and *Eichhornia crassipes*) to evaluate the effect of temperature on the performance of these macrophytes species in a post-treatment system

of swine wastewater. These species have been reported as efficient plants for treating wastewater and pollutants from different sources, such as aquaculture, industry, and domestic sewage (Henry-Silva et al., 2008; Shah et al., 2015). Sudiarto et al. (2019) conducted a study using swine effluent with different aquatic plants and found satisfactory results for the removal of total nitrogen (TN) and total phosphorus (TP). Xu and Shen (2011) and Muradov et al. (2014) also found positive results. During winter and summer, specifically, we attempted to answer the following questions: (i) How does temperature influence (in winter and summer) TN and TP removal from this wastewater by two species of macrophytes? (ii) How do the values of hydrogen ion concentration (pH), electrical conductivity (EC), and dissolved oxygen (DO) vary during the study period (summer and winter)? (iii) How does biomass productivity of both species of macrophytes vary in different temperatures between the seasons? It is expected to find greater efficiency in TN and TP removal by macrophyte species. Moreover, it is expected to find higher biomass and values of electrical conductivity (EC) and dissolved oxygen (DO) in the summer (period with higher temperatures).

Materials and Methods

Field station and experimental systems

The experiment was carried out in the municipality of Toledo, Paraná State, Brazil (24°40'57" S, 53°49'25" W, altitude of 760 m). The study site was built with wooden beams and covered with transparent polyethylene (approximately 0.150 mm thick), preventing the entry

of rain and allowing access to light (Figure 1A). The experiment was carried out in the summer (Feb and Mar) and winter (Aug and Sept) of 2008 (30 days for each experiment). For the experimental system, 24 concrete tanks with 80 L capacity were used, each measuring approximately 1.5 m long, 0.25 m wide, and 0.16 m deep. Each system comprised three tanks arranged at different height levels (0.30 m), totaling eight systems, with four of them for *P. stratiotes* and four for *E. crassipes* (Figures 1B and 1C). The system was operated in open circuit (continuous flow) and wastewater flowed through the three tanks by slope (gravity). Wastewater was stored in drums arranged above the tanks, where effluent was sent to the system continuously.

Plant species

Eichhornia crassipes (Mart. Solms) is native to the Amazon region. It has high growth rates and is considered a cosmopolitan plant (Chandra and Gerhardt, 2008). It can increase its green mass by 15 % in a day, doubling it every six to seven days, under optimal condition, and producing up to 480 tons of green mass per hectare in a year (Barbieri and Esteves, 1991). *E. crassipes* presents better development in tropical climate and can survive low temperatures, even severe winters. Some factors, such as low light intensity, air temperatures between 26 and 30 °C, long photoperiods and high plant density may promote anatomical modifications (Shu et al., 2014). The ideal water temperature for *E. crassipes* growth is between 28 and 30 °C, while low air humidity from 15 % to 40 % may also be a limiting factor for the species growth (Jafari, 2010).

Pistia stratiotes is very common in lentic environments. It has a high potential for development in eutrophic environments and its growth is accelerated under the influence of light incidence in open environments (Farnese et al., 2014). It does not withstand cold or frost; however, its seeds survive freezing temperatures and prefer water with acid to neutral pH and organic background (Hall and Okali, 1974). *Pistia*

stratiotes is a pioneer species, has good development with decomposed organic material, and can be considered an indicator of eutrophication. It is aggressive, fast-moving and thickens mainly in polluted, nutrient-rich water. It is considered a tropical and subtropical species. In Brazil, the species is abundant and widely distributed, both in natural aquatic ecosystems and in aquatic environments affected by anthropic activities (Pott and Pott, 2000). The ideal water temperature for *P. stratiotes* growth is around 15 to 35 °C (Neuenschwander et al., 2009).

Studies published in 1975 and 1999, which used aquatic plants to treat water and effluents, pointed to the removal of N and P nutrients, followed by the removal of metals. These plants are known for their ability to accumulate pollutants, which may occur by physicochemical interaction or by mechanisms dependent on metabolism. The DO amount in the nearest plant layers is reduced, creating anaerobic conditions, maximizing nutrient removal (Azubuike et al., 2016).

Wastewater characteristics and variables measurements

The effluent used was preventient from swine breeding in the municipality of Toledo (24°40'57" S, 53°49'25" W, altitude of 760 m), Paraná State, Brazil. The system originates from the treatment of swine wastewater, known as Biosystem Integration (BSI). The BSI had two biodigesters, a sedimentation tank, two algae tanks, and a tank for pisciculture. The wastewater was collected in the algae rearing tank. After preliminary testing, the swine wastewater effluent was diluted 3:1 with rainwater collected and stored in 1000 L boxes nearby the experimental system, avoiding the phytotoxic effects on plant species, ensuring their survival.

Initial and final physical and chemical analyses of the wastewater performed in triplicate for each experimental system, in both study periods (summer and winter) following standard methods (APHA, 2005). The analysis of total nitrogen (TN) and total phosphorus

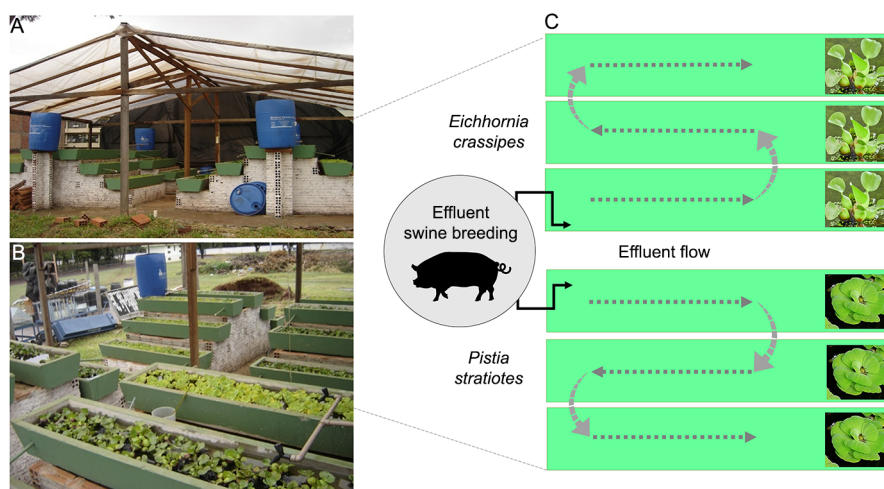


Figure 1 – A and B) Experiment figures and C) Scheme of the experimental system used in the study.

(TP) was determined at the beginning and at the end of the experiments. We used the method of nitroperchloric digestion and sulfuric digestion following the methodology described by (Golterman et al., 1978). Hydrogen potential (pH), electrical conductivity (EC), and dissolved oxygen (DO) were measured weekly. For pH determination, a digital pH meter was used. The amount of DO was determined by a portable oximeter and EC by a portable conductivity meter.

The air and effluent temperatures were measured at 8:00 a.m., 12:00 p.m., and 4:00 p.m. on each day of the experiment using mercury thermometers with scale from -10 to + 60 °C and division of 1 °C. The minimum temperature recorded during the experimental periods was measured using a chapel type thermometer.

Aquatic macrophytes and centesimal composition

The aquatic macrophytes used were the Water hyacinth (*Eichhornia crassipes* (Mart.) Solms) and Water lettuce (*Pistia stratiotes* L.). The plants were collected at the Aquaculture Research Center - CPA (in the municipality of Toledo) and transported to the experiment site in water tanks to avoid water stress. Afterwards, the plants were deposited in 25,000-gallon tanks, remaining for 30 days for the biological adaptation of the species in the new environment (Espinoza-Quinonês et al., 2005). We evaluated some chemical properties of the plants before subjecting them to the treatment system. The analyses were repeated at the end of the experiment, according to the analytical standards of the Association of Official Analytical Chemists (AOAC, 2000). Three repetitions were made for each parameter. The methods used for the analysis were: percentage of crude protein (%CP) by the Kjeldahl method using the N transformation factor in protein of 6.25, ref. 9402 (AOAC, 1996), humidity (%) at 105 °C until reaching constant weight, ref. 650.56 (AOAC, 1996), ash (%) muffle incineration at 550 °C, ref. 93808 (AOAC, 1996), nitrogen (N) in Kjeldahl digestion (AOAC, 2000), and phosphorus (P) (Barbieri and Esteves, 1991) that were expressed as g kg⁻¹. Each concrete tank received 2.0 kg of plants, totaling 6.0 kg per system, which were removed from the 25 m³ water

tank and deposited on a screen. Therefore, excess water was drained. After the experimental period, all plants were removed and weighed to obtain the total biomass gain.

Statistical analysis

The experimental design was completely randomized, composed of two species of macrophytes, arranged in four replications, and performed in two seasons (summer and winter). Data were submitted to analysis of variance (ANOVA) and t-test (parametric data). The Tukey test was applied when the ANOVA showed significant differences, always considering a level of significance of 5 % of probability. The PAST software version 3 was used for statistical analysis. For the statistical comparisons, it was evaluated if the temperature averages varied in relation to the season, then if this variation affected efficiency of aquatic macrophytes for N and P removal between seasons. Next, we compared the means of electric conductivity (EC) and dissolved oxygen (DO) between the seasons. Finally, we evaluated biomass gain of the aquatic macrophytes between the seasons and their chemical composition.

Results

The average air temperatures (minimum and maximum) differed significantly between the seasons (summer and winter), as well as the average wastewater temperature in the three evaluations ($p < 0.05$; Figures 2A-C). Therefore, we evaluated the temperature effect on nitrogen (TN) and phosphorus (TP) removal by the aquatic macrophytes in the different seasons. In the initial condition, the wastewater had an average contents of 403.1 mg L⁻¹ for NT and 54.4 mg L⁻¹ for PT (Table 1). The results showed high efficiency in TN and TP removal by both species evaluated. The highest TN efficiency was observed in the summer, with 88 % removal for *Pistia stratiotes* and 80 % removal for *Eichhornia crassipes*. According to Tchobanoglous et al. (2003), this high removal percentage may also be related to the slight alkalinity observed in the effluent, as the pH was above 7, ammonium ions (NH₄⁺) can be volatilized as ammonia

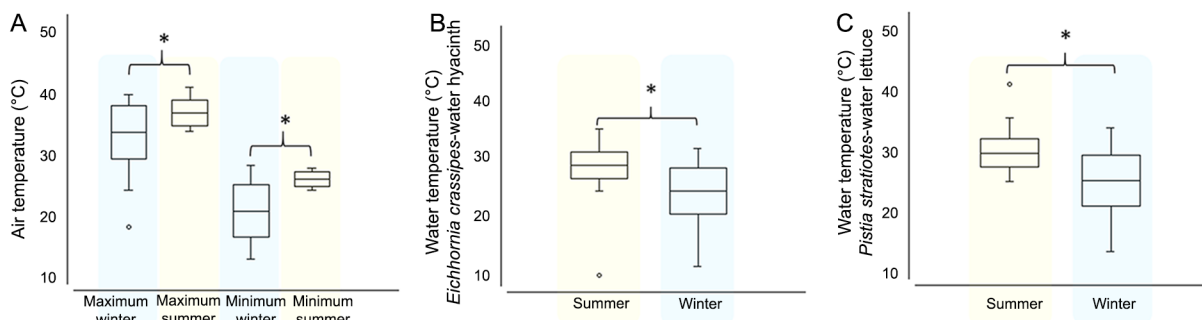


Figure 2 – A) Box plot (average and deviations) showing the variation in air temperature during the experiment period (summer and winter of 2008); B) Wastewater temperature of *Eichhornia crassipes* in summer and winter and C) Wastewater temperature of *Pistia stratiotes* in summer and winter. *Indicates significant differences. *Significant difference ($p \leq 0.05$) between treatments during the summer and winter periods.

(NH₃⁺), contributing to TN removal of the effluent. For TP, *Eichhornia crassipes* had greater removal in the winter (39 %) differing from *Pistia stratiotes*, which had greater removal in the summer (38 %, Table 1).

The pH, EC and DO variables presented different results in relation to the initial wastewater values. The initial pH values were slightly alkaline (within the range 8.2), after the treatment with the aquatic macrophytes, the pH reduced significantly for both species ($p < 0.05$). Regarding the pH and the periods of evaluation, there was a significant difference between the summer and winter ($p < 0.05$, Table 2). The decrease in EC was also significant between treatments and evaluated periods, with better results for winter in both wastewater assessments (Table 2). Finally, DO contents presented an addition of 2.7 mg L⁻¹ of DO in the summer, significantly different from the winter period for *Eichhornia crassipes* ($p < 0.05$). However, for *Pistia stratiotes*, the final DO values for winter and summer did not differ significantly. In relation to the chemical composition of the aquatic macrophytes, although some

variables were significantly different between the periods of the experiment, in general, most analyzed variables in the aerial part (AP) and root (R) did not present significant differences between the periods studied (Table 3).

The initial weight of natural biomass for the treatments and year periods was 2.00 kg m⁻² per tank, totalling 24 kg m⁻² in each treatment system for each period. The final biomass production differed statistically ($p \leq 0.008$) between the periods for the treatment with *E. crassipes* and did not present difference in the treatment with *P. stratiotes*. Biomass production for *E. crassipes* was 67.2 % in the summer and 59.2 % in the winter, while *P. stratiotes* presented biomass gain of 50 % in the summer and 28.6 % in the winter (Table 4).

Discussion

Air temperatures observed in this study showed that the plants remained for long periods under lower temperatures during the winter. Water temperature

Table 1 – Initial contents of the wastewater, average values, and standard deviation of the final contents and percentage (%) of nitrogen (TN) and phosphorus (TP) removal by treatment systems with *Eichhornia crassipes* and *Pistia stratiotes* in the summer and winter periods.

Parameters	Initial wastewater	<i>Eichhornia crassipes</i> (water hyacinth)		<i>Pistia stratiotes</i> (water lettuce)	
		Summer final	Winter final	Summer final	Winter final
NT contents (mg L ⁻¹)	403.1 ± 22.3	78.9 ± 27.3*	156.7 ± 69.8	49.0 ± 35.5*	191.9 ± 68.1
Removal (%)		80 ± 6.8	61.1 ± 17.3	88 ± 8.8	52.4 ± 17
PT contents (mg L ⁻¹)	61.23 ± 4.8	40.4 ± 3.8*	37.5 ± 3.2	38.1 ± 4.0	39.3 ± 0.7*
Removal (%)		34.1 ± 6.2	38.8 ± 5.2	37.6 ± 6.3	35.8 ± 1.2

*Significant difference ($p \leq 0.05$) between treatments during the summer and winter periods.

Table 2 – Average values and standard deviation of pH, electrical conductivity (EC), and dissolved oxygen (DO) at the beginning and during the experimental phase (30 days) by the macrophytes in the summer and winter period.

Treatment	pH			CE (µS cm ⁻¹)			DO (mg L ⁻¹)		
	Initial	Summer final	Winter final	Initial	Summer final	Winter final	Initial	Summer final	Winter final
<i>Eichhornia crassipes</i>	8.2 ± 0.2	7.6 ± 0.3*	7.4 ± 0.2	2.6 ± 0.5	1.6 ± 0.4*	1.3 ± 0.5	1.3 ± 0.5	4.0 ± 1.4*	1.4 ± 1.1
<i>Pistia stratiotes</i>	8.2 ± 0.2	7.7 ± 0.3*	7.6 ± 0.3	2.6 ± 0.5	1.6 ± 0.4*	1.4 ± 0.5	1.3 ± 0.5	2.8 ± 1.3 ns	1.4 ± 1.0

*Significant difference ($p \leq 0.05$) between treatments during the summer and winter periods. ns = not significant.

Table 3 – Initial and final average and standard values of humidity, mineral matter, crude protein (CP), and macronutrients (phosphorus and nitrogen) in the total biomass (dry matter) in the aerial part (AP) and root (R) of *Eichhornia crassipes* and *Pistia stratiotes* periods of summer and winter.

Parameters	Treatment					
	<i>Eichhornia crassipes</i>			<i>Pistia stratiotes</i>		
	Initial	Summer	Winter	Initial	Summer	Winter
Humidity (AP)	10.5 ± 0.1	8.6 ± 1.5*	6.4 ± 0.8	11.7 ± 0.1	6 ± 0.9 ns	6.8 ± 0.9
Humidity (R)	8.6 ± 0.7	4.7 ± 0.7 ns	5.7 ± 0.8	9.1 ± 0.2	4.7 ± 1.3 ns	4.9 ± 0.6
Crude protein (AP)	17 ± 3.5	30.3 ± 2.6 ns	34.5 ± 5.3	11 ± 1.8	28.5 ± 3.3 ns	29.6 ± 3
Crude protein (R)	12 ± 2.3	28.8 ± 3.2*	24.4 ± 3.8	10 ± 2.1	25.3 ± 2.2 ns	26.5 ± 5
Ashes (AP)	29.4 ± 7.3	18.9 ± 2.5	22.6 ± 3.7*	22.8 ± 2.9	23.1 ± 1.4 ns	23 ± 1.9
Ashes (R)	17.2 ± 5.3	16.2 ± 2.6	21.8 ± 3.6*	22.3 ± 2.1	23.9 ± 1.1	27 ± 3.8*
Phosphorus (AP) (g kg ⁻¹)	14.9 ± 0.2	29.1 ± 4.7	50.5 ± 6.6*	14.9 ± 0.3	26.8 ± 6.5	41.9 ± 7.1*
Phosphorus (R) (g kg ⁻¹)	16.5 ± 0.5	38.1 ± 5.6 ns	37.6 ± 8.1	18 ± 0.6	30.4 ± 5.3	40.4 ± 7.6*
Nitrogen (AP) (g kg ⁻¹)	13.4 ± 1.5	89.3 ± 7.7*	38.9 ± 3.1	12.2 ± 1.1	85.8 ± 6*	33.8 ± 3.2
Nitrogen (R) (g kg ⁻¹)	15.3 ± 1.6	68.3 ± 22.9*	26.4 ± 3.8	12.6 ± 3.9	65.6 ± 13*	32.4 ± 2.3

*Significant difference ($p \leq 0.05$) between treatments during the summer and winter periods. ns = not significant.

Table 4 – Initial and average weight of the final biomass weight in the treatment systems with *Eichhornia crassipes* and *Pistia stratiotes* in the summer and winter periods. Average values and standard deviation of biomass produced, and daily biomass gain after 30 days of treatment.

Parameters	Treatment			
	<i>Eichhornia crassipes</i>		<i>Pistia stratiotes</i>	
	Summer	Winter	Summer	Winter
Initial weight (kg m ⁻²)	2.00	2.00	2.00	2.00
Final weight (kg m ⁻²)	6.1	4.9	4.0	2.8
Biomass Production (kg m ⁻²)	4.1 ± 1.6*	2.9 ± 0.6	2.0 ± 1.4ns	0.8 ± 1.1
Daily biomass gain (g m ⁻² d ⁻¹)	136 ± 0.1*	95 ± 0.02	70 ± 0.05*	25 ± 0.04

*Significant difference ($p \leq 0.05$) between treatments during the summer and winter periods. ns = not significant.

was below the ideal range for *E. crassipes* and within the tolerable values for *P. stratiotes*. In this scenario, there was significant difference between the winter and summer in all the hours of the day when temperatures were measured. In relation to TN and TP removal, these components are responsible for eutrophication of water bodies (Mees et al., 2009). Nitrogen is involved in the formation of proteins, basic components of biomass. Under low concentrations, N may act as a limiting factor in the primary production of aquatic ecosystems (Feresin et al., 2010). Phosphorus can also act as a limiting factor in the primary production of aquatic ecosystems, leading to eutrophication. This fact may lead to changes in the physical, chemical, and biological properties of the environment, causing losses in productivity and biodiversity (Li et al., 2018; Mees et al., 2009).

The wastewater used in this research had high TN and TP contents, which are highly polluting if released directly into the environment. The aquatic plant species used in this research had a high potential for the removal of these nutrients, corroborating previous studies (González et al., 2009; Mees et al., 2009). According to Van de Moortel et al. (2010), N removal is more strongly influenced by season and temperature, because these factors affect mainly the activity of microorganisms. In our study, temperature had a direct influence on N absorption, since average air and water temperatures were lower in the winter. According to Mitsch and Gosselink, 1993, at low temperatures, biological processes slow down dramatically or cease. The presence of plants in humid areas results in more stable temperatures, which may promote the removal of pollutants (Hill and Payton, 2000). Some studies indicate a relationship between N and P contents in water and aquatic plants (Li et al., 2018; Nogueira et al., 1996; Petracco, 1997). The high N and P contents in the wastewater are much higher than the values allowed by Res.357/05 - MMA (2005), which establishes values of 2.18 mg L⁻¹ for N and 0.1 mg L⁻¹ for P, when released in lotic environments. The biomass increase observed shows that this index, even high, did not prevent biomass accumulation, without interfering in the vegetative growth of the *E. crassipes* in the summer. Finally, regarding TN and TP removal, both macrophytes showed high efficiency in TN removal and accumulation of wastewater in both experiment periods. It is possibly related to the primary production and the

high N contents in plants (Shah et al., 2015; Sooknah and Wilkie, 2004).

Both species of macrophytes used in this research were capable of reducing the pH values after the treatment in both periods evaluated. This reduction was expected due to the biochemical requirements related to the vegetative growth of macrophytes, which can modify the aquatic environment through oxidation of the rhizosphere, proton excretion (H⁺), release of organic acids and carbon dioxide (CO₂) (Armstrong et al., 1990; Tanner et al., 1995; Coleman et al., 2001). The values found after the treatment by macrophytes in both periods were within the standards established by federal legislation, 6.0 to 9.0 Res. 357/05 - Brazil (2005). However, the EC levels observed in this study do not represent a risk for the growth of both macrophytes. The results indicate efficiency in reducing the EC in relation to the initial concentration, indicating a reduction in the availability of dissolved salts in the effluent. Regarding DO, several authors have reported that high water temperatures also reduce the capacity to keep DO at optimum levels (Ishida et al., 2006; Mamais and Jenkins, 1992).

In relation to the bromatological characteristics of macrophytes, after the treatment, there was an increase in their biomass for most parameters evaluated when compared to the initial values. Our results show that temperature influenced the moisture content in the samples of the aerial part of *E. crassipes*, presenting a higher percentage during the summer. Temperature had no influence on the tissues of *P. stratiotes*. This DM reduction in all treatments may be related to the high N contents in wastewater. Nitrogen is an important constituent of proteins and is directly related to increases in the primary production of aquatic macrophytes (Thomaz et al., 2006).

The percentage of crude protein (CP) was influenced by temperature in the root samples of *E. crassipes* during the summer. There was a significant difference in CP percentages in the summer when compared to the winter samples. *Pistia stratiotes* did not influence this variable when analyzed statistically between both periods. *Eichhornia crassipes* and *P. stratiotes* could be considered as plants with high protein content because they present CP values above 12 % (Boyd, 1970). According to Henry-Silva et al. (2001), plants that store more minerals tend to contribute more directly to nutrient cycling. Thus, during the winter,

when temperatures were lower, *E. crassipes* had a higher percentage of mineral matter in its tissues. Differently, for *P. stratiotes*, mineral matter was influenced only in the root samples. However, the mineral matter found in the biomass of aquatic macrophytes is one of the main limiting factors for its use as fodder. This high ash content tends to decrease OM content, reducing the nutritive value of these plants. Therefore, the lower the mineral content of a species, the greater its contribution in terms of OM production. Our results show that *E. crassipes* and *P. stratiotes* presented a high ash content.

Conclusion

The use of aquatic macrophytes were efficient for final swine wastewater polishing, since macrophytes showed efficiency in the removal of N and P. The TN removal showed higher efficiency in the summer for both species studied. *Eichhornia crassipes* showed greater TP removal in the winter, different from the behavior presented by *P. stratiotes*. The abiotic variables, pH and EC presented better results during the winter, for both species and were not influenced by the low air and wastewater temperatures. Dissolved oxygen presented the best results in the summer for *E. crassipes*. The species studied showed a significant increase of N and P in plant tissue. The high CP levels found in the species studied indicate their high potential for use in animal feed and composting. Finally, in this study, *E. crassipes* was more efficient than *P. stratiotes* in the treatment of swine wastewater effluent.

Acknowledgements

To Jéssica Manfrin for help in the English language. The 1st and 4th authors thank the Coordination for the Improvement of Higher Level Personnel (CAPES) for granting the PhD scholarship and the 3rd author was supported by CAPES through a post-doctoral scholarship (88887.318827/2019-00). This study was financed in part by the CAPES - Finance Code 001.

Authors' Contributions

Conceptualization: Weirich, C.E.; Feiden, A.; Klosowski, E.S.; Souza, C.S. Data acquisition: Weirich, C.E.; Feiden, A.; Klosowski, E.S. Data analysis: Weirich, C.E.; Souza, C.S. Design of methodology: Weirich, C.E.; Feiden, A.; Klosowski, E.S. Writing and editing: Weirich, C.E.; Feiden, A.; Klosowski, E.S.; Marchetti, C.R.; Aleixo, V.; Souza, C.S.

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