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Research paper

Influence of sediment on self-fertilization by total phosphorus in mesocosmos simulating phytoremediation of eutrophic aquatic environment

Influência do sedimento na autofertilização por fósforo total em mesocosmos simulando a fitorremediação de ambiente aquático eutrofizado

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ABSTRACT: To restore a eutrophic aquatic ecosystem, it is essential to eliminate external sources of nutrients. Furthermore, the sediment compartment can store large amounts of nutrients, which can be released into the water column and keep the ecosystem eutrophic for a long time, even after the removal of external sources of nutrients. In this sense, the present study aimed to evaluate, in mesocosms, the influence of sediment in self-fertilizing an aquatic environment with phosphorus and its consequence in delaying the recovery of this environment if a phytoremediation technique with *Eichhornia crassipes* was used. The study was conducted in closed-bottom mesocosms using water, macrophytes, and sediment from a hypereutrophic reservoir (Lago das Garças, São Paulo, SP). Two types of systems were evaluated: with and without sediment. The study was carried out in triplicate, totalling six mesocosms of 500 L each. Eight monitoring campaigns were carried out between Feb and Jun/2017. The following water quality parameters were determined: pH, electrical conductivity, turbidity, apparent color, and total phosphorus (TP). Plant management was carried out to maintain only half of the colonized free surface and allow the reproduction and growth of the plants. Although both systems showed a tendency to decrease the TP content in the water, it was noted that the system with sediment presented greater reproduction of *E. crassipes* individuals, suggesting the influence of sediment in the self-fertilization of the system. Thus, it is important to consider the sediment when restoring eutrophic environments.

Keywords: bioremediation, ecological engineering, eutrophication, macrophytes, water hyacinth.

RESUMO: *A fim de restaurar um ecossistema aquático eutrofizado, é essencial eliminar as fontes externas de nutrientes. Além disso, o compartimento sedimento pode estocar grandes quantidades de nutrientes, os quais podem ser liberados para a coluna d'água e manter o ecossistema eutrofizado por muito tempo, mesmo após a eliminação das fontes externas de nutrientes. Nesse sentido, o presente estudo teve como objetivo avaliar, em mesocosmos, a influência do sedimento em autofertilizar um ambiente aquático com fósforo e sua consequência no retardamento da recuperação deste ambiente caso fosse empregada uma técnica de fitorremediação com Eichhornia crassipes. O estudo foi conduzido em mesocosmos de fundo fechado utilizando água, macrófitas e sedimento de reservatório hipereutrófico (Lago das Garças, São Paulo, SP). Foram avaliados dois tipos de sistemas: com e sem sedimento. O estudo foi realizado em triplicata, totalizando seis mesocosmos de 500 L cada. Foram realizadas 8 campanhas de monitoramento entre fev. e jun./2017. Foram determinados os seguintes parâmetros de qualidade da água: pH, condutividade elétrica, turbidez, cor aparente e fósforo total. Foi realizado o manejo das plantas a fim de manter apenas a metade da superfície livre colonizada e permitir a reprodução e o crescimento das plantas. Apesar de ambos os sistemas terem apresentado tendência de diminuição dos teores de fósforo total na água, notou-se que o sistema com sedimento apresentou maior reprodução dos indivíduos de E. crassipes, sugerindo a influência do sedimento na autofertilização do sistema. Assim, é importante considerar o sedimento na restauração de ambientes eutrofizados.*

Palavras-chave*: aguapé, biorremediação, engenharia ecológica, eutrofização, macrófitas.*

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INTRODUCTION

Artificial eutrophication of aquatic ecosystems is one of nowadays main environmental problems (UNESCO, 2018; JENNY et al., 2020). This phenomenon consists of increasing the primary productivity (algae and aquatic plants) of water bodies and it is triggered by increased concentrations of nutrients (nitrogen and phosphorus) (GUO et al., 2014). Human actions, such as discharge of untreated or treated sewage without a specific stage of nutrient removal, greatly contribute to the intensification of this process (TRINDADE; MENDONÇA, 2014; BALDOVI et al., 2021). Eutrophication has several undesirable consequences, such as: depletion of dissolved oxygen levels, fish death, proliferation of potentially toxic algae, decreased biodiversity, increased greenhouse gas emissions and increased costs of water treatment for human consumption (GUO et al., 2014; TRINDADE; MENDONÇA, 2014; BEAULIEU; DEL SONTRO; DOWNING, 2019; COELHO et al., 2020; BENASSI et al., 2021).

Various cases of restoration of eutrophic aquatic environments have been reported in the literature (JIANBO et al., 2008; GUO et al., 2014; CHEN et al., 2020). Remediation techniques of eutrophic aquatic environments, in addition to being highly expensive, may involve the use of chemicals causing risks to the microbiota and preventing nutrient recovery (HUISMAN et al., 2018). In this sense, solutions based on nature, such as phytoremediation with aquatic macrophytes can be a green and low-cost alternative to control the eutrophication of aquatic ecosystems (CHEN et al., 2020), and also enables the restoration of resources (nutrients, use of biomass for energy production and civil construction, among others), and stimulates the circular economy (GUO et al., 2014).

One of the widely used macrophyte species is *Eichhornia crassipes*, commonly found in tropical regions and which has the ability to fix chemical elements in their tissues in quantities above their needs, which configures it as a good alternative for use in remediation of eutrophic environments (COELHO, 2017). As an example, Pistori (2009) concluded that *E. crassipes* has a better performance regarding nutrient absorption compared to *Salvinia molesta* and *Pistia stratiotes*, both in low and high concentrations of nutrients. Analogously, in a study conducted in microcosm with sediment, Wang et al. (2017) observed that *E. crassipes* presented better performance than *P. stratiotes* in improving quality parameters in the treatment of aquaculture effluents. In parallel with these aspects, other recent studies have reported the ability of *E. crassipes* to remove phosphorus from water (e.g. SUNG et al., 2015; SU et al., 2019; BALDOVI et al., 2021).

It is emphasized that, in order to restore a eutrophic aquatic ecosystem, it is essential to control the external sources of nutrients (SCHINDLER et al., 2008; SCHINDLER, 2012). That said, even after the external sources cease, the sediment compartment can maintain the system eutrophic for many years due to nutrient stocks (SCHINDLER, 2012; SØNDERGAARD et al., 2013, TU et al., 2019), which can be released into the water column under certain pH and oxygenation conditions (BICUDO et al., 2007).

Given what has been said, the present study aimed to evaluate, in mesocosmos, the influence of sediment on self-fertilizing an aquatic environment with phosphorus and its consequence in delaying the recovery of this environment if a phytoremediation technique with *Eichhornia crassipes* had been used.

MATERIAL AND METHODS

The study was conducted near Garças Lake (23° 38'S, 46° 37'W) which is located in the Parque Estadual das Fontes do Ipiranga (PEFI), in São Paulo, SP. This aquatic ecosystem has a surface area of 88,156 m², an average depth of 2.1 m, a maximum depth of 4.7 m, and a theoretical mean residence time of the water of 71 days (BICUDO et al., 2007). The climate in the region is tropical of high altitude (CONTI; FURLAN, 2003). The latosols are predominantly in the PEFI. The Garças Lake was eutrophicated throughout the 20th century due to the release of sewage from the Zoological Garden Foundation in São Paulo (FPZSP), and the Secretary of Agriculture and Supply (SAA) (BICUDO et al., 2007; COSTA-BÖDDEKER et al., 2012).

Currently, the SAA drains entry no longer exists; and the FPZSP treats its sewage; howbeit, without a specific stage of nutrient removal. Thus, the sediments generated in Garças Lake come from the discharge of effluents from the FPZSP, as well as from the decomposition of the biomass of algae, aquatic plants and other organisms generated from the metabolism of the lake itself. The experiment consisted of two systems: without sediment (Control) and with sediment (Treatment), in triplicates, resulting in six experimental units. **Figure 1** presents the experimental setting and **Figure 2** illustrates some steps in the experiment.

Figure 1. Experimental setup of phytorremediation with *Eichhornia crassipes in mesocosms:* Control (mesocosms without sediment, with water and macrophytes, n = 3) and Treatment (mesocosms with water, sediment and macrophytes, n=3)

The experiment was set up on February 13 of 2017. Six polypropylene water tanks with 500 liters of capacity each (Dimensions: D = 1200 mm and H = 800 mm) were used. The tanks were covered with anti-mosquito netting to prevent the proliferation of insects on the site. Three tanks indicated as "Control" were filled with water and individuals of the species *E. crassipes* both extracted from the Garças Lake. Another three tanks called "Treatment" where filled with 8 liters of lacustrine sediment, water and macrophytes from Garças Lake. The sediment was collected using a Eckman's Dredger. Water and sediment were collected in the pelagic region of the reservoir; the macrophytes were collected from a macrophyte bank in the Garças Lake and transferred directly to the mesocosmos, in order to evaluate the influence of the sediment on the delay of the phytoremediation process by *E. crassipes*.

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Figure 2. Images of the experimental units: (a) six mesocosms of 500 liters of capacity each, located close to Garças Lake; (b) tanks with sediment during installation of mesocosms (c) tanks with water of the lake and *Eichhornia crassipes* (d) tanks were covered with anti-mosquito nettings

The system was monitored from February 15, 2017 to June 1, 2017, totaling 8 samplings. The pH and electrical conductivity were monitored *in situ* by a multiparameter probe (Hanna, model HI 9829) at three distinct points in the water subsurface of each tank. Water samples (~300 mL) were collected on the subsurface using polyethylene bottles. It was tried, as much as possible, to prevent the movement of water in order to avoid sediment resuspension, which could interfere with the results. In the sequence, the samples were transported in Styrofoam boxes with ice for further analysis in the laboratory. In some of the samples, some macrophyte individuals were removed in order to maintain only about 50 to 60% of the water surface colonized, avoiding to limite plant development by space (BENASSI et al., 2018).

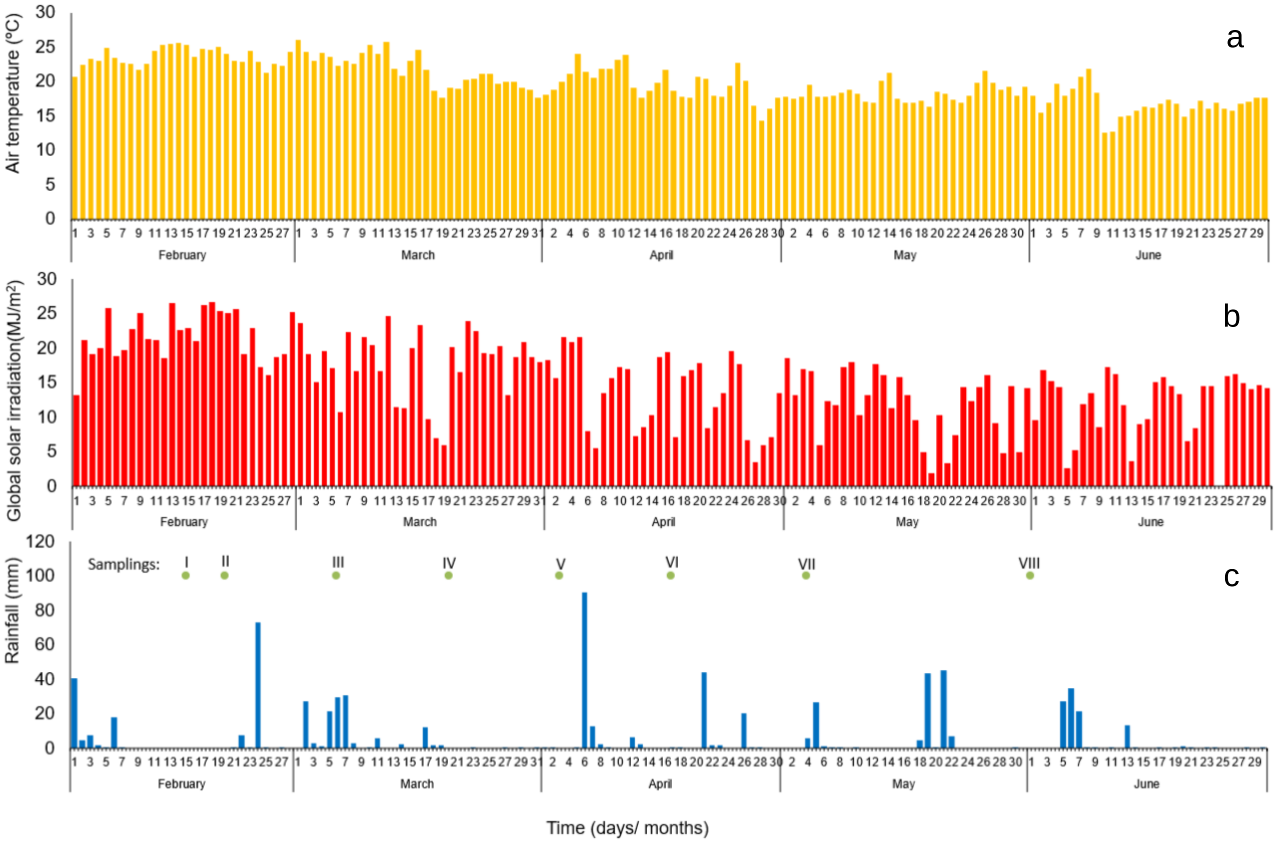
Other analytical determinations were performed in the laboratory: apparent color, using a photo colorimeter (Policontrol); turbidity, by means of a turbidimeter (Policontrol) and total phosphorus (TP) concentration, by acid digestion with potassium persulfate, followed by colorimetric determination with ascorbic acid and reading in spectrophotometer (HACH, model DR 5000), according to APHA (2012). Climatic data (air temperature, solar irradiation, relative humidity, and precipitation) were obtained from the Meteorological

Station of the Astronomical and Geophysical Institute of the University of São Paulo, located about 500 m from the site of installation of the experimental units.

RESULTS AND DISCUSSION

The air temperature and solar irradiation showed a tendency to decline over the experimental period (**Figures 3a** and **3b**), which was expected, since the study started in the summer and was completed in late autumn. Precipitation peaks during the experiment period were recorded in late February and early April (**Figure 3c**).

Figure 3. Climatic variables throughout the experimental period: (a) Air temperature (°C); (b) Global solar irradiation (MJ m^{-2}); (c) Rainfall (mm) and samplings (green dots)



The results of the water quality variables are presented in **Figure 4**. The pH ranged from 4.8 to 8.7 throughout the study (**Figure 4a**). The highest pH values were recorded at the beginning of the experiment. High pH values are typical of eutrophic aquatic ecosystems due to the assimilation of CO_2 dissolved in water by algae during the photosynthesis process (BICUDO et al., 2007, TAILING, 2010; BRASIL et al., 2016). This alters the balance of the carbonate system, which decreases the production of carbonic acid and increases the pH from the water, which can cause phosphorus precipitation (BAIRD, 2002). From the third sampling (March 6), the pH values were always below 6.6, on which phosphorus precipitation was not expected.

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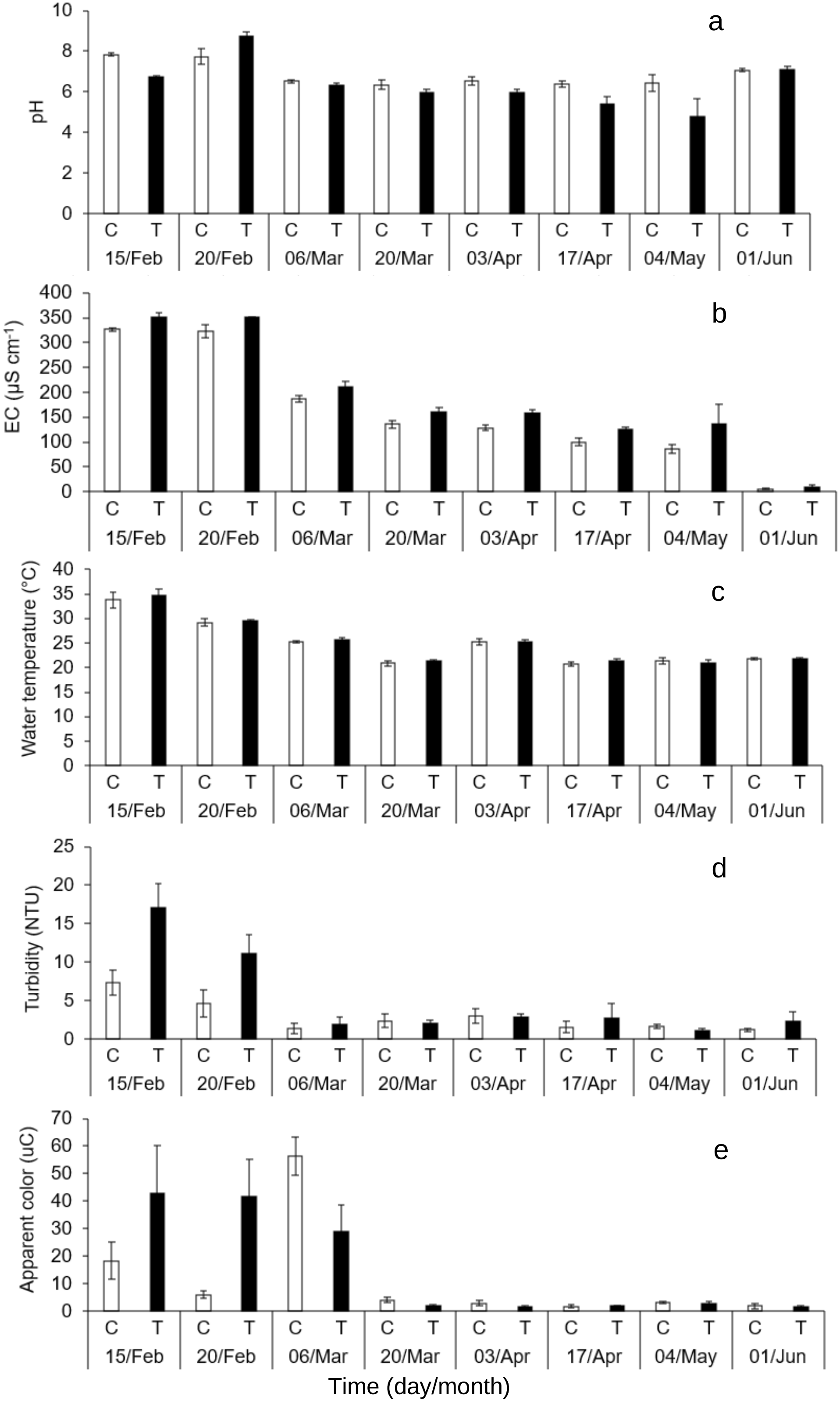
Figure 4. Average values and standard deviation of water quality variables in mesocosms without sediment (Control, "C") and with sediment (Treatment, "T") throughout the study period: (a) pH; (b) Electric Conductivity, EC (μS cm^{-1}); (c) water temperature; (d) turbidity (NTU); (e) apparent color (uC)



The electrical conductivity (EC) showed a decreasing trend throughout the experiment (**Figure 4b**). In the first two samples, the EC values were above 300 µS cm^{-1}, typical of polluted environments (COELHO et al., 2020). From the third sampling (March 6), the EC values showed a decreasing trend, reaching 6.13 µS cm^{-1} in the last sample in the tank without sediment; and 10.41 µS cm^{-1} in the tank with sediment. It was noticed that the EC values were always slightly higher in tanks with sediment, probably due to the release of dissolved ions from the sediment towards the water column. Rain events may have influenced the results of the study and, probably, caused a dilution effect. However, this is a limitation of the mesocosmos technique. Though, despite the limitations, mesocosmos are extremely useful, since they allow studying on a smaller scale and in a more controlled way the processes that occur in aquatic environments, as well as testing remediation techniques (AMARAL et al., 2020; HAOKIP; GUPTA, 2020).

The water temperature (**Figure 4c**) followed the same downward trend observed for air temperature, due to seasonal variation. Yet, the lowest value recorded was 20.7 ºC, which is a value considered adequate for the development of *E. crassipes* (DUKE, 1983). Regarding turbidity, higher values were noted in tanks with sediment in the initial samples, with a large reduction for both systems along the other samples, reaching 1.18 NTU (**Figure 4d**), which demonstrates water clarification. The highest turbidity values in tanks with sediment at the beginning of the experiment may represent a stabilization phase of the experiment. As for the apparent color, there was a tendency to decrease it throughout the experiment, reaching 1.6 uC; except in sample III for the Control tank, where an increase in this variable was recorded (**Figure 4e**). Color is associated with the amount of substances dissolved in water, which may have colloidal characteristics that change it (BÁRBARA; CUNHA; SIQUEIRA, 2010).

The TP contents in the water column decreased throughout the observation period, with a small increase in sample IV (March 20) for both systems. It is noteworthy that, from sample VI on (April 17), the recorded averages were below 3 µg L^{-1} of TP for both systems (**Figure 5**). This may reflect the assimilation by the biota (microalgae and macrophytes), as well as sedimentation with the particulate matter, since there was also a decrease in turbidity.

Figure 5. Average values and standard deviation of total phosphorus concentrations (µg L^{-1}) in mesocosms without sediment (Control, "C") and with sediment (Treatment, "T") throughout the monitoring period

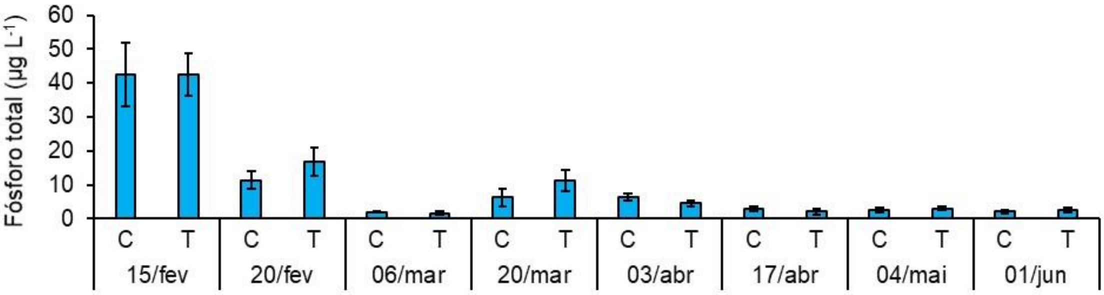
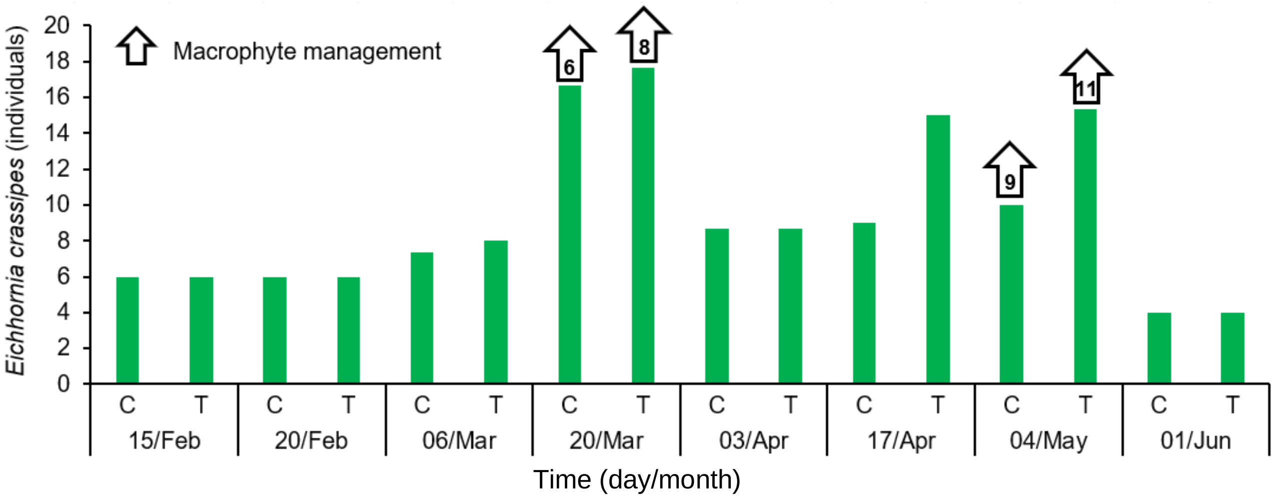


Figure 6 illustrates the development of *E. crassipes* biomass through the number of individuals and their management throughout the experimental period. There is an increase in the number of individuals from collection III, with a peak in collection IV, where

the first handling was carried out (removal of individuals). After that, the individuals proliferated again, with a record of growth and increase in the number of individuals and a new peak in collection VII, where the second management was carried out. It was noted that the number of *E. crassipes* individuals was slightly higher in the tanks with sediment in collections VI and VII (1.5 - 1.7 times), suggesting self-fertilization by phosphorus from the sediment and incorporation into the biomass. In fact, in collection IV (20/03), the concentrations of PT in the water were almost double in the tanks with sediment (11.3 µg L^{-1} against 6.2 µg L^{-1}) and the EC was always slightly higher. high in ponds with sediment.

Figure 6. Average values of number of *Eichhornia crassipes* individuals in mesocosms without sediment (Control, "C") and with sediment (Treatment, "T") throughout the monitoring period. Up arrows indicates the numbers of individuals removed (management)



High air temperature and solar irradiation favor the development of the species *E. crassipes* (BEYRUTH, 1992) and this is due to the increase in metabolic activity of aquatic organisms (YOSHIDA, 1996). In fact, the optimum temperature range for the development of this species is 25 °C to 31 °C (PEDRALLI, 1996). In the present study, despite the variations in temperature and solar irradiation, satisfactory plant development was noticed, with an increase in the number of individuals (**Figure 6**). Gentelinni et al. (2012) observed similar performance, where temperatures ranged between 18.4 °C and 19.1 °C and this did not significantly affect the development of individuals of *E. crassipes*, although maximum growth was not achieved.

Regarding pH, as in the initial samples there was greater availability of nutrients, the predominant effect was alkalization of the water, justified by the assimilation of carbon dioxide dissolved in water by algae and aquatic macrophytes during photosynthesis (BICUDO et al., 2007) and alteration of carbonate system balance (BAIRD, 2002).

The decrease in EC values may be associated with the absorption of ions present by individuals of *E. crassipes*, since this variable represents the concentration of dissolved ions (ESTEVES, 1998). This is corroborated by the decrease in TP concentration in the systems throughout the experiment (**Figure 5**). As expected, Coelho et al. (2020) found significant positive correlations between TP concentrations and electrical conductivity for eutrophic spring waters (Rio Grande Reservoir, São Paulo). Besides, precipitation may have affected EC, since the higher water intake may lead to a proportional decrease in dissolved ions, which characterizes a dilution effect as recorded by Pompêo et al. (1996)

when evaluating the effect of macrophyte *Echinochloa polystachya* on Jurumirim Dam, in São Paulo.

The decrease in turbidity in the tanks is associated with the great development of the macrophyte roots throughout the study, which adsorbed the particulate matter in suspension (HENRY-SILVA; CAMARGO, 2008). Other factor that justifies the decrease in turbidity is the absence or minimal occurrence of resuspension.

Perhaps if the experiment had been carried on, the tanks with sediment could have remained productive. As suggestions for future studies, it is highly recommended to monitor the system for a longer period, as well as to evaluate the production of periphyton on the sediment (epipelon), which has been pointed out as an important factor in the maintenance of low phosphorus concentrations in water (CANO; CASCO; CLAPS, 2016; ZHAO et al., 2019; YI et al., 2019). On top of this, it is paramount to monitor the dissolved oxygen indices, since oxygenation indices in the tanks may be responsible for the retention of phosphorus in the sediment or its release to the water column, reported in recent studies (HORPILLA, 2019; LIU et al., 2020).

CONCLUSIONS

There was a significant decrease in TP concentrations in both mesocosmos evaluated (presence and absence of sediment), which can be attributed to removal by macrophytes (phytoremediation), assimilation by microalgae and sedimentation with particulate matter. Notwithstanding, a higher number of individuals of *E. crassipes* was noted in tanks with sediment (reaching 1.7 times), which suggests the influence of sediment on the self-fertilization of tanks with phosphorus, which could delay an effort to recover the eutrophic aquatic environment.

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