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Short Communication

Tolerance of free-floating aquatic macrophytes to sugarcane vinasse and its implications for phytoremediation strategies

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ABSTRACT

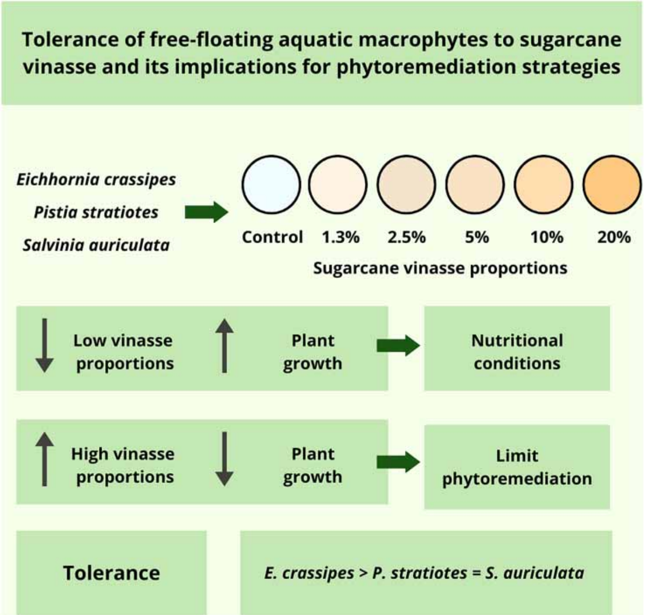
Sugarcane vinasse is a byproduct of the ethanol industry that has been associated with environmental impacts, including ecotoxicity. However, several nature-based alternatives have been studied to attenuate environmental pollution, including phytoremediation. In such a scenario, this research aimed to evaluate the phytotoxicity of vinasse to aquatic plants, assessing the tolerance of three free-floating aquatic macrophytes, namely *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia auriculata*. Five vinasse proportions (up to 20%) were considered to assess the short-term growth of aquatic plants. *P. stratiotes* and *S. auriculata* were more sensitive to vinasse than *E. crassipes*, which showed resistance at 10 and 20%. Lower dilutions were non-toxic and even stimulated the growth of macrophytes, which might contribute to the uptake of nutrients and potentially toxic elements. The selection of tolerant species contributes to the application of phytotechnologies, and the studied plants might have potential for the phytoremediation of vinasse.

Key words: agricultural residues, aquatic macrophytes, ecotoxicology, green manure, soil quality, water quality

HIGHLIGHTS

- Investigating nature-based solutions like phytoremediation can help address sugarcane vinasse pollution.
- Low proportions of vinasse had effects on short-term plant growth, including growth stimulation.
- High proportions of vinasse inhibit the growth of aquatic plants and limit their application for phytoremediation.
- *Eichhornia crassipes* was the most tolerant species, even up to 20% of vinasse.

GRAPHICAL ABSTRACT



INTRODUCTION

The expansion of sugarcane cultivation and processing is associated with environmental impacts that require further study (Christofoletti *et al.* 2013; Ogura *et al.* 2022b). The ethanol industry produces significant amounts of sugarcane vinasse, a byproduct of distillation, with 10–18 L generated for each liter of ethanol (Silva *et al.* 2007). Vinasse has been used in fertigation due to its high concentration of organic matter (OM) and nutrients, such as N, P, K, Ca, and Mg. However, this alternative has been linked to soil salinization, acidification, and phytotoxicity in cases where inadequate application exceeds recommended doses (Silva *et al.* 2007; Christofoletti *et al.* 2013). Such a byproduct can contaminate water resources through runoff and leaching, leading to acidification, increased turbidity, and imbalanced dissolved oxygen (DO) levels (Gunkel *et al.* 2007; Ogura *et al.* 2022a; Pinto *et al.* 2023). The ecotoxicity effects of vinasse in water have been studied on various species from different niches, including fish (Correia *et al.* 2017), crustaceans (Botelho *et al.* 2012), and oligochaetes (Souza *et al.* 2022).

Mitigating the environmental impacts of vinasse can contribute to restoring ecological balance, and nature-based solutions stand out as promising techniques. Phytoremediation with aquatic plants provides a natural, sustainable, and effective approach to remediate contaminated waters (Khan *et al.* 2023; Latif *et al.* 2023). Aquatic macrophytes can be used, and these organisms are essential for maintaining ecosystem functionality. The potential of plant-based solutions for remediating sugarcane vinasse was already evidenced in constructed wetlands (Marcato *et al.* 2019). Nonetheless, the phytotoxicity of vinasse might limit the phytoremediation capacity, which brings the relevance of assessing plant growth and tolerance when exposed to varying vinasse proportions.

This study aimed to evaluate the limits of phytotechnologies based on the short-term phytotoxicity of sugarcane vinasse on three aquatic plant species, namely *Eichhornia crassipes* Mart. (Pontederiaceae), *Pistia stratiotes* L. (Araceae), and *Salvinia auriculata* Aubl. (Salviniaceae). The macrophytes were exposed to five different dilutions of vinasse for 7 days, and the assessed endpoints included the number of fronds, fresh biomass, and root growth.

METHODS

The study was carried out at the Nucleus of Ecotoxicology and Applied Ecology (NEEA) at the University of São Paulo (USP) (Itirapina, São Paulo, Brazil). The sugarcane vinasse used in this research was collected at a sugarcane mill in the region (Porto Ferreira, São Paulo, Brazil). The sample was collected on 2 September 2019 and was stored in a freezer (approximately −15 °C) until the experiments (in the following 6 months). The physical and chemical characterization of the study vinasse followed the procedures described in Method 3111B Standard Methods for the Examination of Water and Wastewater (APHA 2018). The parameters included pH, electrical conductivity (EC), DO, and nutrients (e.g., total nitrogen and phosphorus). Other elements were analyzed through direct atomic absorption spectrophotometry (AA 240FS VARIAN), including aluminum, barium, cadmium, lead, cobalt, copper, chromium, strontium, manganese, molybdenum, nickel, potassium, and zinc.

The macrophytes *E. crassipes*, *P. stratiotes*, and *S. auriculata* were grown under ambient conditions in 1,500 L tanks with non-contaminated groundwater and a supply of nitrogen, phosphorus, and potassium. The macrophytes were disinfected with sodium hypochlorite (at 1%, for 1 min) to eliminate algae and were washed three times with distilled water. The organisms were acclimated at 25 °C for 7 days before each experiment in a Hoagland's solution at 10% (Hoagland & Arnold 1950).

The experimental design consisted of non-toxic 1 L plastic containers, and the tests had three replicates ($n = 3$) of a laboratory control (without contamination) and five vinasse dilutions (1.3, 2.5, 5, 10, and 20%). For *E. crassipes*, selected organisms had four to six fronds and a 3 cm root length. For *P. stratiotes*, organisms had four to six fronds, and from 2 to 3 cm roots were selected per unit. For *S. auriculata*, each organism had eight fronds and root lengths between 3 and 5 cm. The organisms of each species chosen for the bioassays had no statistical differences in their initial parameters (i.e., root length, fronds, and fresh biomass) among all replicates. The tests were carried out in a room with controlled temperature (25 ± 2 °C), illuminance (800 lx), and photoperiod (12 h: 12 h). The assays lasted for 7 days without medium renewal, following the adaptation of the *Lemna* sp. growth inhibition test (OECD 2006). The parameters pH, EC, and DO were measured throughout the experiment with a compound sample of all replicates. After 7 days of exposure, the endpoints of growth or inhibition were the number of fronds, fresh biomass, and root lengths.

Data analyses were conducted using Statistica version 7 software. The normality and homogeneity of the data were assessed using the Shapiro–Wilk and Levene tests, respectively. Non-parametric data were subsequently transformed as necessary. Statistical differences ($p < 0.05$) between treatments were determined using analysis of variance (ANOVA) with a 95% confidence level, followed by post hoc tests using Dunnett and Tukey methods. The effects of vinasse were compared across different proportions and the control, considering the number of fronds, fresh biomass, and root length.

RESULTS

Table 1 shows the physical and chemical characterization of the raw vinasse. The study vinasse had low pH (4.12) and DO (0.72 mg L^{-1}). On the other hand, the raw vinasse showed high conductivity (11,400 µS cm^{-1}) and nutrient concentrations, including nitrogen (146.53 mg L^{-1}), phosphorus (17.23 mg L^{-1}), and potassium (3,749.00 mg L^{-1}). Al and Mn were the only potentially toxic metals with concentrations higher than 1.0 mg L^{-1} (16.59 and 2.24 mg L^{-1}, respectively).

Initially, the addition of vinasse decreased the pH of all samples (from 6.01 up to 4.09), although they became more neutral (from 6.03 to 7.67) after 7 days. The initial DO concentrations were not altered by the contamination with vinasse; however, the values substantially decreased after 7 days (up to 0.39 mg L^{-1} at 10%). EC increased as the dilutions were higher, which is attributed to the high concentrations of elements from the raw vinasse composition. Figure 1 shows a summary of the

Table 1 | Raw vinasse physical and chemical characterization

Parameter	Raw vinasse	Parameter	Raw vinasse
pH	4.12	Cobalt (mg L^{-1})	<LD
Conductivity (µS cm^{-1})	11,400	Copper (mg L^{-1})	0.438 ± 0.002
Dissolved oxygen (mg O_2 L^{-1})	0.72	Chromium (mg L^{-1})	0.041 ± 0.001
Total nitrogen (mg L^{-1})	146.530	Strontium (mg L^{-1})	0.571 ± 0.002
Total phosphorus (mg L^{-1})	17.285	Manganese (mg L^{-1})	2.239 ± 0.040
Aluminum (mg L^{-1})	16.587 ± 0.357	Molybdenum (mg L^{-1})	0.006 ± 0.002
Barium (mg L^{-1})	0.098 ± 0.000	Nickel (mg L^{-1})	<LD
Cadmium (mg L^{-1})	<LD	Potassium (mg L^{-1})	3,749.000 ± 71.500
Lead (mg L^{-1})	0.084 ± 0.003	Zinc (mg L^{-1})	0.731 ± 0.004

Note: LD refers to the limit of detection for each method, equivalent to 0.0006, 0.007, and 0.008 mg L^{-1} for Cd, Co, and Ni, respectively. The concentrations were presented as mean values along with their corresponding standard deviations.

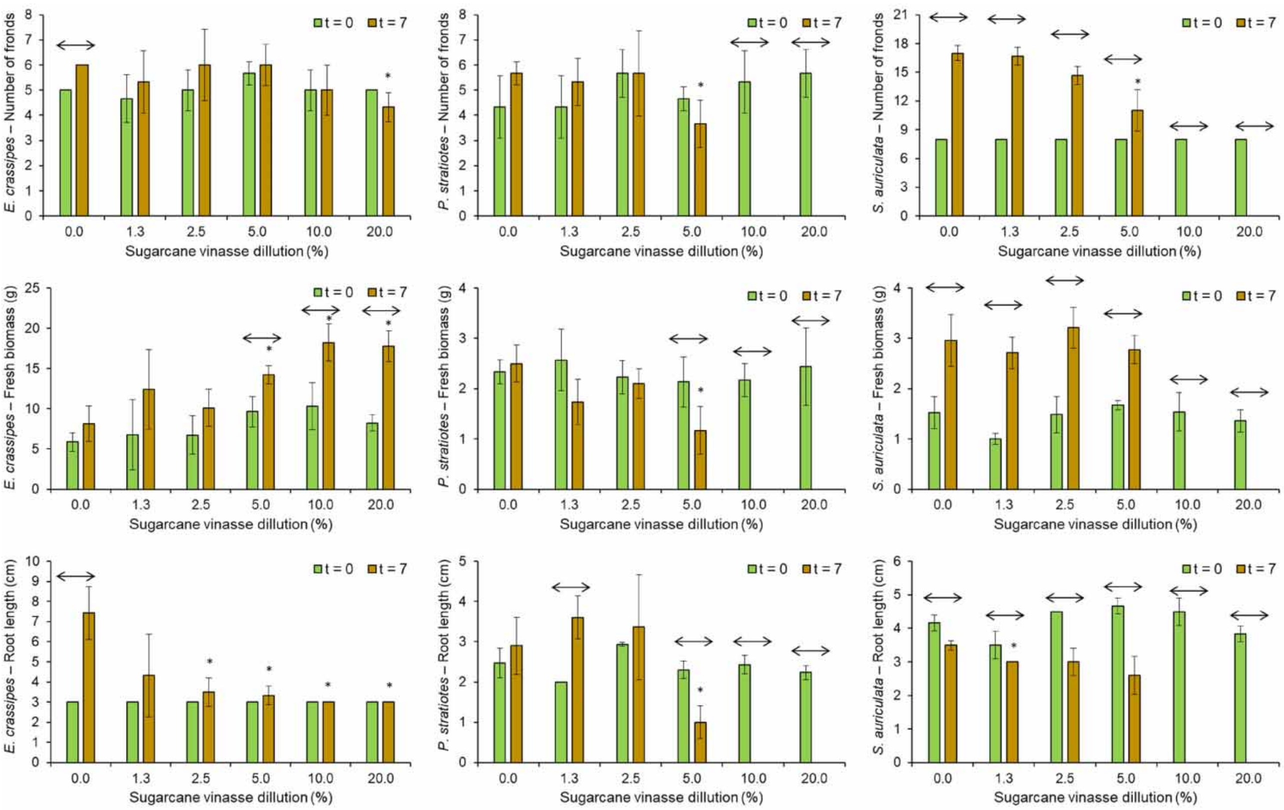


Figure 1 | Number of fronds, fresh biomass, and root lengths of *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia auriculata* exposed to five dilutions of sugarcane vinasse for 7 days ($t = 0$ and $t = 7$). *Notes:* bars represent the average and standard deviation; arrows over bars indicate differences ($p < 0.05$) within the same treatment from $t = 0$ and $t = 7$; asterisks represent the difference from the control ($p < 0.05$).

phytotoxicity results, including the number of fronds, fresh biomass, and root lengths of the studied macrophytes exposed to sugarcane vinasse for 7 days. The studied organisms of each species did not show statistical differences ($p > 0.05$) at the beginning of the tests.

For *E. crassipes,* there were differences in the number of fronds, fresh biomass, and root length after 7 days ($p < 0.05$). The growth in the number of fronds at 20% (4.3 ± 0.6) was lower than the control (6.0 ± 0.0) ($p < 0.05$). The highest vinasse dilutions (from 5 to 20%) promoted an increase in the fresh biomass (control = 8.1 ± 2.2 g; other dilutions = from 10.1 to 18.2 g) ($p < 0.05$), despite the root growth being significantly higher in the control group (7.4 ± 1.3 cm) than the other dilutions (from 3.0 to 4.3 cm). However, there was a significant decrease in the number of fronds, fresh biomass, and root length at 5% of vinasse for *P. stratiotes* (3.6 ± 0.9, 1.2 ± 0.5 g, and 1.0 ± 0.4 cm, respectively) and *S. auriculata* (11.0 ± 2.2, 2.8 ± 0.3 g, and 2.6 ± 0.6 cm, respectively) ($p < 0.05$). Among all treatments, only these two species reported lethality at 10 and 20%. For *P. stratiotes*, the other vinasse dilutions were similar to the control ($p > 0.05$). *Salvinia auriculata* showed a significant reduction in root growth even at 1.3% (3.5 ± 0.1 cm in the control, and 3.0 ± 0.0 at 1.3%) ($p < 0.05$). The macrophytes at 2.5 and 5% showed burns and frond deformations, indicating sub-lethal effects of vinasse.

DISCUSSION

Vinasse has the potential to decrease the water DO due to its high organic content and biochemical oxygen demand (Gunkel *et al.* 2007; Ogura *et al.* 2022a). It influences water quality, especially by its toxicity to the aquatic biota and high concentrations of solids and nutrients (Pinto *et al.* 2023). Although nutrients (e.g., N, P, and K) contribute to the growth of aquatic plants, anthropogenic contributions to water bodies might be associated with artificial eutrophication (Moran-Salazar *et al.* 2016). The low pH of vinasse might enhance its phytotoxicity. In Botelho *et al.* (2012), a reduction in vinasse toxicity (in terms of LC_{50}) was achieved after a pH adjustment (from 4 to 7) to the cladocerans *Ceriodaphnia dubia* (from 0.67 to 2.99%) and *Daphnia magna* (from 0.8 to 5.62%), besides the fish *Danio rerio* (from 2.62 to 8.34%).

The low pH of raw vinasse (4.12) and its high metal concentrations (e.g., Al and Mn) can explain the growth impairments of macrophytes. The presence of Al (16.59 mg L^{-1}) and Mn (2.24 mg L^{-1}) in the raw vinasse induces its phytotoxicity, as observed for the biomass growth inhibition of the macrophyte *Ricciocarpos natans* L. (Al from 1.5 to 5.0 mg L^{-1}, and Mn at 3.0 mg L^{-1}) (Gimenes *et al.* 2020). Concentrations of Al above 0.3 mg L^{-1} were toxic to the submerged macrophyte *Vallisneria natans*, reducing the biomass and root weight, besides the number of plants and the average plant height (Lin *et al.* 2020). As sugarcane vinasse is composed of several elements, their potentially toxic effects might be enhanced in combination. However, vinasse generally shows variable physical and chemical parameters in the literature, which is a challenge for comparing its ecotoxicological effects across several studies (Botelho *et al.* 2012; Christofoletti *et al.* 2013; Garcia *et al.* 2017; Coelho *et al.* 2018; Pinto *et al.* 2023).

Pires *et al.* (2016) applied the *Allium cepa* bioassay to evaluate the phytotoxicity of raw vinasse (at 20, 40, 60, and 80%) and observed a decrease in the germination at higher proportions; vinasse at 20% inhibited seed germination by 26.3% and root length 28.8% compared to the control. At this same dilution in our study, only *E. crassipes* showed a higher tolerance to sugarcane vinasse, while the other two species of aquatic plants did not survive. In other studies, raw vinasse completely inhibited the germination of *A. cepa* due to low pH and the presence of potentially toxic elements (Pedro-Escher *et al.* 2016), and non-lethal effects were observed for this species with chromosomal alterations when exposed to 2.5 and 5.0% of vinasse (Garcia *et al.* 2017). Although vinasse at 1.3% did not show evident effects on the tested macrophytes, irrigation with water containing 1.3% of similar vinasse (i.e., collected from the same industry) inhibited the development of the plants *Phaseolus vulgaris* L. and *Zea mays* L. (up to 31% inhibition), contrary to the expected growth stimulus (Ogura *et al.* 2022a).

Understanding the phytotoxic effects of vinasse can contribute to define the limits of remediation studies. Marcato *et al.* (2019) evaluated a hybrid system on a mesocosm-scale composed of the stabilization of raw vinasse in a pond (for 147 days) and a filtration process (20 μm filter paper), followed by the phytoremediation with *E. crassipes* (for 7 and 15 days); these authors reported that macrophytes were able to assimilate nitrogen and potassium from the sugarcane vinasse, especially in the plant roots. However, phytoremediation of raw vinasse without a pre-treatment might be impaired by the phytotoxicity at higher dilutions, as shown for our studied plants. Lu *et al.* (2018) studied the feasibility of a phytoremediation system for rural rivers that receive domestic sewage and observed that *E. crassipes* and *P. stratiotes* removed phosphorus and nitrogen and reduced chemical oxygen demand. Therefore, these species might also be potentially applied to recover nutrients from other effluents. Considering the potential application of aquatic macrophytes in constructed wetlands, the definition of the hydraulic retention time is relevant for feasibility, as prolonged periods may be impractical on a larger scale due to the associated cost-ineffectiveness and the requirement for increased structural capacity.

CONCLUSIONS

Sugarcane vinasse is a potential contaminant in sugarcane cultivation and processing areas, and its effects on aquatic and terrestrial biota should be further evaluated. The macrophytes *P. stratiotes* and *S. auriculata* were more sensitive to vinasse than *E. crassipes*, which showed resistance at 10 and 20%. Although low dilutions did not cause effects of phytotoxicity to the studied macrophytes, other aquatic organisms might be endangered by vinasse contamination. In our study, the bioassays focused on the short-term effects (7 days) of vinasse on free-floating aquatic macrophytes, but future studies can evaluate these effects for extended periods aimed at assessing their application in constructed wetlands. The low number of tested organisms, the high standard deviation values, and the lack of quantification of nutrient uptake were limitations of our research. Future studies should evaluate the potential of these plants for the phytoremediation of vinasse, especially with *E. crassipes*, the most tolerant of the studied aquatic plants, including the analysis of bioaccumulation of nutrients and potentially toxic metals. Studies should also address the management and destination of the plant biomass after the remediation, which is a challenge for the application of phytotechnologies.

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AUTHOR CONTRIBUTIONS

L. F. P. B. investigated the data and wrote the original draft; E. L. G. E. wrote the review and edited the article, arranged the resources, and rendered support in funding acquisition; A. P. O. conceptualized the whole article, developed the methodology, rendered support in formal analysis, investigated the article, wrote the original draft, reviewed and edited the article, supervised the work, and administered the project.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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