

Effect of Urucu oil (Brazilian Amazon) on the biomass of the aquatic macrophyte *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae)

Efeito do petróleo de Urucu (Amazônia/Brasil) sobre a biomassa da macrófita aquática *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae)

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Abstract: The rivers and floodplain lakes of the Urucu petroleum-bearing region in central Amazonia are extensively colonized by aquatic macrophytes, which may be affected by accidental spills during oil extraction and transportation operations. Among the species of macrophytes, the free-floating *Eichhornia crassipes* (water hyacinth) is abundant; **Aim:** The objective of this study was to evaluate the effect of different dosages of Urucu crude oil (0, 0.5, 1.5 and 3.0 L.m⁻²) on the biomass of *E. crassipes* and on some physical and chemical characteristics of the water in which the plant grew; **Methods:** The experiment was conducted over a period of 84 days. Every seven days, the biomass (live and dead) of *E. crassipes* and the temperature, pH, electrical conductivity and dissolved oxygen in the water were measured; **Results:** The dosage of 0.5 L.m⁻² was sufficient to cause partial mortality (48%) in *E. crassipes* after 35 days of exposure to oil. The dosage of 3.0 L.m⁻² caused total mortality (100%) in *E. crassipes* after 84 days of exposure. The decomposition of the oil and the dead biomass of *E. crassipes* reduced the dissolved oxygen and pH, and increased electrical conductivity and concentrations of total phosphorus in the water; **Conclusions:** We conclude that an oil spill can cause total mortality in the local population of one macrophyte species, but not of another. This may alter the macrophyte species diversity in the impacted region. In the case of *E. crassipes* and *Pistia stratiotes*, an Urucu crude oil spill could favor *E. crassipes*, the species less sensitive to oil.

Keywords: water, water hyacinth, biomass, dosage, mortality.

Resumo: Os rios e lagos de várzea da província petrolífera de Urucu, na Amazônia Central, são amplamente colonizados por macrófitas aquáticas, que podem ser afetadas por acidentes durante a exploração e o transporte de petróleo. Entre as macrófitas, a espécie flutuante *Eichhornia crassipes* (aguapé) ocorre abundantemente na região; **Objetivo:** O objetivo desse estudo foi verificar o efeito de diferentes dosagens do petróleo de Urucu (0; 0,5; 1,5 e 3,0 L.m⁻²) na biomassa viva e morta de *E. crassipes* e em algumas características físicas e químicas da água; **Métodos:** O experimento teve oitenta e quatro dias de duração. A cada sete dias foi determinada a biomassa (viva e morta) de *E. crassipes* e os valores de temperatura, pH, condutividade elétrica e oxigênio dissolvido da água; **Resultados:** A dosagem de 0,5 L.m⁻² foi suficiente para causar mortalidade parcial (48%) em *E. crassipes* após trinta e cinco dias de exposição ao petróleo. A dosagem de 3,0 L.m⁻² causou mortalidade total (100%) em *E. crassipes* em oitenta e quatro dias de exposição. A decomposição do petróleo e da biomassa morta de *E. crassipes* provocam a redução do oxigênio dissolvido e do pH, e aumento da condutividade elétrica e de fósforo total na água; **Conclusões:** Nós concluímos que um derramamento de petróleo pode provocar mortalidade total em uma população de uma espécie de macrófita, mas não em uma outra. Isto pode alterar a diversidade de espécies de macrófitas na região impactada. No caso de *Eichhornia crassipes* e *Pistia stratiotes*, um derramamento de petróleo de Urucu pode favorecer *E. crassipes*, a espécie menos sensível ao petróleo.

Palavras-chave: água, aguapé, biomassa, dosagem, mortalidade.

1. Introduction

In the Urucu region of the Brazilian Amazon, intense exploitation and transportation of oil and natural gas are continuing. The Urucu field produces 50 thousand barrels of oil and 10.36 thousand cubic meters of natural gas per day (Petrobrás, 2010). This production generates a risk of accidental spills, which could seriously impact rivers and floodplain lakes. The crude oil extracted from the Urucu field is classified as 'light', and according to Pezeshki and DeLaune (1993), this type of petroleum can be easily taken up by vegetation and cause immediate harm.

Aquatic macrophytes are an important community in shallow-water ecosystems, possessing a high rate of primary production, participating in nutrient recycling and providing food for herbivores and detritivores (Tundisi and Matsumura-Tundisi, 2008). Floodplain lakes of the Amazon region are rich in aquatic macrophytes, including the abundant free-floating water hyacinth *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) (Lopes et al., 2009). This species reproduces sexually and asexually, and can rapidly colonize new areas, forming dense banks where conditions are favorable (Villamagna and Murphy, 2010).

The impacts of an oil spill on the aquatic vegetation depend on diverse factors, including i) the characteristics of the oil, its type, degradation time and how its components alter over the period when it remains in contact with the vegetation; ii) the vegetation aspects, such as the species, the parts of the plants involved, the growth and phenological stages; and iii) the environmental conditions, such as sediment granulometry, season of the year, inundation level and climate characteristics (Lin and Mendelssohn, 1996; DeLaune et al., 2003).

The majority of studies on the effects of petroleum products on aquatic macrophytes have involved species of estuarine regions (Lin and Mendelssohn, 1996; Stepanyan and Voskoboinikov, 2006; Wernick et al., 2009), and similar studies of freshwater macrophytes are rare. In Brazil, Silva and Camargo (2007) showed that *Pistia stratiotes* L. (Araceae) is extremely sensitive to Urucu petroleum; low doses (0.1, 0.2 and 0.3 L.m⁻²) caused death in plants. The effect of Urucu oil on *E. crassipes* was evaluated by Lopes et al. (2009), who observed that exposure of this species to concentrations between 0.08 and 15.89 L.m⁻² reduced the number and size of leaves. The objective of the current study was to evaluate the effects of different dosages of Urucu

crude oil on the biomass of *E. crassipes*, through the determination of live and dead biomass of the whole plant. In addition, we evaluated the alterations in the physical and chemical characteristics of the water that were caused by the exposure of the macrophyte to oil.

2. Material and Methods

An experiment with four treatments and three replications was conducted over 84 days in the Experimental Garden of the Biosciences Institute (UNESP, Rio Claro) in the State of São Paulo, Brazil. Each treatment consisted of a different oil dosage: 0 (control), 0.5, 1.5 and 3.0 L.m⁻². The oil dosage used in the treatments was based on other studies such as those of Pezeshki and DeLaune (1993), Pezeshki et al. (1998), Silva and Camargo (2007) and Lopes et al. (2009). The 12 experimental units consisted of plastic boxes 0.55 × 0.35 m (a surface area of 0.1925 m²) and 0.25 m deep; the boxes were filled initially with 630 ± 17 g wet mass of *E. crassipes* per m² (gWM.m⁻²). The size of the boxes and the mass of the macrophyte were used to simulate a nearshore area of a typical waterbody. The individuals of *E. crassipes* were collected in natural freshwater habitats in southern São Paulo State, Brazil, where the species is abundant. The Urucu crude oil was obtained from the Urucu Oil Province, through the Training Program of Human Resources in Petroleum Geology and Environmental Sciences Applied to Oil & Gas and Biofuels (PRH-05) of Unesp/Rio Claro. The experimental units were initially filled with water, then *E. crassipes* was added, and finally the oil.

Every seven days, the temperature, pH, electrical conductivity and dissolved oxygen were measured in the water of each experimental unit, using a Horiba U-10 multisensor. The water level of the boxes was also completed after the measurements. The total phosphorus (TP) (Golterman et al., 1978) and total Kjeldahl nitrogen (TKN) (MacKereth et al., 1978) concentrations in the water of each experimental unit were measured at the start and end of the experiment.

At weekly intervals, the live and dead biomass of *E. crassipes* was measured. We defined dead biomass as that with more than 50% colored brownish-yellow, and the tissues detached from the individual. Live biomass was removed from the experimental units, placed in sieves for approximately 5 minutes to drain excess water (Agami and Reddy, 1990), weighed (as gWM.m⁻²), and returned to the respective experimental unit. Dead biomass was

also removed from the experimental units every seven days, oven-dried to constant weight and then weighed (gDM.m⁻²). The dead material was not returned to the experimental units, and the weights obtained every seven days were summed. The live wet biomass was transformed to grams of dry mass (gDM.m⁻²) by a linear regression between dry mass (DM) and wet mass (WM) of plants that were not used in the experiment, but originated from the same population as the plants used in the experiment. The equation obtained was $DM = 0.92876 + 0.06299 \cdot WM$ ($r = 0.99$). Water and *E. crassipes* data were analyzed to verify normality and homoscedasticity using the D'Agostinho and Bartlett tests. Subsequently, repeated-measures ANOVA and, if required, a Tukey test were applied to identify significant differences ($P \leq 0.05$) between the water quality variables (pH, electrical conductivity and dissolved oxygen) and between the live biomass of *E. crassipes* in the four treatments (0, 0.5, 1.5 and 3.0 L.m⁻²) in different weeks (time). One-way ANOVA and, if required, a Tukey test were used to identify significant differences ($P \leq 0.05$) between the TP and TKN concentrations in water of the four treatments at the end of the experiment.

3. Results

Repeated-measures ANOVA indicated effects of the oil dosage and time on the pH, electrical conductivity and dissolved oxygen of the water (Table 1). The initial pH was equal in all experimental units, but on day 84, the pH was significantly lower in the control (4.46 ± 0.35) and higher in the dosages of 1.5 L.m⁻² (6.16 ± 0.15) and 3.0 L.m⁻² (6.36 ± 0.5). Electrical conductivity showed a significant increase between days 0 and 84 in all treatments. On day 84, electrical conductivity was significantly lower in the control (0.077 ± 0.009 mS.cm⁻¹) and higher in 3.0 L.m⁻² (0.167 ± 0.007 mS.cm⁻¹). Dissolved oxygen differed significantly between the treatments on day 0. On this day, the value recorded for the control was 4.41 ± 0.70 mg.L⁻¹, and for 3.0 L.m⁻² was only 0.70 ± 0.26 mg.L⁻¹. Subsequently, the concentrations of dissolved oxygen in 0.5, 1.5 and 3.0 L.m⁻² remained significantly lower than in the control until day 84 (Figure 1). The water temperature in the experimental units varied between 22.3 and 30.2 °C during the experiment.

On the first day of the experiment in all treatments, the TKN and TP levels were 0.016 ± 0.003 mg.L⁻¹ and 45.3 ± 8.1 µg.L⁻¹,

respectively. TKN did not differ significantly among the treatments at the end of the experiment (day 84) ($F = 0.97$, $P > 0.05$). TP values were significantly different on day 84 ($F = 18.21$, $P \leq 0.05$). The highest value was found in the dosage of 3 L.m⁻² and the lowest in the control (Table 2). Repeated-measures ANOVA revealed effects of the oil dosage and time on the live biomass of *E. crassipes* (Table 3). The live biomass in the control treatment (0 L.m⁻²) increased significantly between day 0 (39.8 ± 3.0 gDM.m⁻²) and day 84 (70.5 ± 7.1 gDM.m⁻²). At the

Table 1. Results of repeated-measures ANOVA applied to data for pH, electrical conductivity and dissolved oxygen of water in the four oil-addition treatments (0, 0.5, 1.5 and 3 L.m⁻²) on different days (time), df = degrees of freedom and MS = mean square.

	gl	MS	F	P
pH				
Treatment	3	10.42	21.17	0.00038
Time	12	1.18	11.03	0.00001
Treatment × Time	36	0.57	5.37	0.00001
Electrical conductivity				
Treatment	3	0.02	35.62	0.00005
Time	12	0.01	237.27	0.00001
Treatment × Time	36	0.01	11.17	0.00001
Dissolved oxygen				
Treatment	3	120.35	107.73	0.00001
Time	10	1.06	6.72	0.00001
Treatment × Time	30	0.43	2.72	0.00020

Table 2. Means and standard deviations (±) of total Kjeldahl nitrogen (TKN) and total phosphorus (TP) of water in the four oil-addition treatments (0, 0.5, 1.5 and 3 L.m⁻²) at the start (day 0) and end (day 84) of the experiment. Different letters indicate significant differences ($P \leq 0.05$) between the treatments by Tukey test.

Treatments	TKN (mg.L ⁻¹)	TP (µg.L ⁻¹)
Beginning	0.016 ± 0.003^a	45.3 ± 8.1^b
0 L.m ⁻²	0.011 ± 0.001^a	21.3 ± 1.4^c
0.5 L.m ⁻²	0.012 ± 0.004^a	$35.9 \pm 22.7^{b,c}$
1.5 L.m ⁻²	0.013 ± 0.003^a	$63.4 \pm 27.9^{a,b}$
3.0 L.m ⁻²	0.017 ± 0.007^a	133.5 ± 11.8^a

Table 3. Results of repeated-measures ANOVA applied to data for live biomass of *Eichhornia crassipes* in the four oil-addition treatments (0, 0.5, 1.5 and 3 L.m⁻²) on different days (time).

	gl	MS	F	P
Live biomass				
Treatment	3	9082.80	18.69	0.00056
Time	12	463.10	12.33	0.00001
Treatment × Time	36	699.40	18.62	0.00001

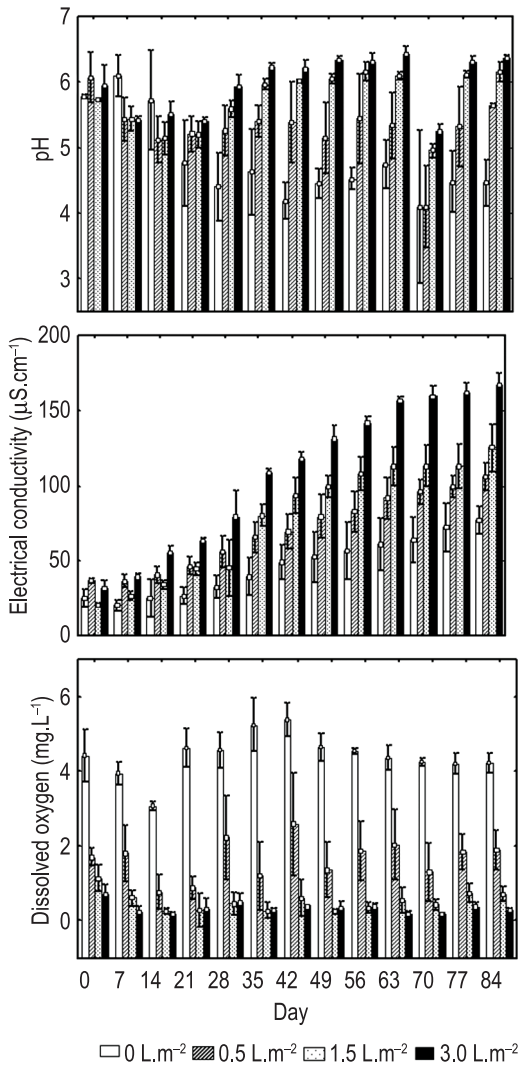


Figure 1. Means and standard deviations of pH, electrical conductivity and dissolved oxygen of water in the four oil-addition treatments (0, 0.5, 1.5 and 3 L.m⁻²).

dosage of 0.5 L.m⁻² live biomass also increased significantly between day 0 (39.8 ± 3.0 gDM.m⁻²) and day 84 (66.7 ± 11.8 gDM.m⁻²). After day 35, the live biomass in the 1.5 L.m⁻² treatment was significantly less than in the control and reached 45.7 ± 5.3 gDM.m⁻² on day 84. For the 3.0 L.m⁻² dose, live biomass was significantly less than in the control after day 35, with total mortality at day 84 (0 gDM.m⁻²) (Figure 2). For the dosage of 0.5 L.m⁻², mortality began on day 35 (7.5 ± 2.7 gDM.m⁻²) and on day 84 the dead biomass value was 21.1 ± 6 gDM.m⁻². For the 1.5 L.m⁻² and 3 L.m⁻² dosages, mortality began after day 21, and on day 84 the dead biomass values were 30.5 ± 11.1 gDM.m⁻² and 70.0 ± 11.1 gDM.m⁻², respectively. No mortality of *E. crassipes* occurred in the control treatment (Figure 2).

4. Discussion

The Urucu crude oil negatively affected the live biomass of *E. crassipes*. A dosage of 0.5 L.m⁻² caused partial mortality, and 3.0 L.m⁻² caused total mortality in this species after 84 days exposure. *E. crassipes* showed lower sensitivity to Urucu crude oil than do other species of Amazon macrophytes. A dosage of 0.2 L.m⁻² caused total mortality of the free-floating macrophyte *Pistia stratiotes* after 91 days of exposure (Silva and Camargo, 2007), and a dosage of 0.66 L.m⁻² caused total mortality of the aerial parts of the emergent macrophyte *Echinochloa polystachya* (H.B.K.) Hitchcock (Poaceae) within 21 days of exposure (Lopes et al., 2009). The smaller size and the haired leaf surface of *P. stratiotes* may partly explain the higher toxicity of oil to this species than to *E. crassipes* (Silva and Camargo, 2007). The characteristics of the leaf of *P. stratiotes* allow oil to be absorbed by the leaf surface. The presence of oil on the surface of the leaves impedes gas exchange, and this affects photosynthesis and plant growth (Pezeshki and DeLaune, 1993). Oil can also alter cell-membrane permeability, thus endangering plant metabolic processes (DeLaune et al., 2003). The lower sensitivity of *E. crassipes* to oil compared to that of *E. polystachya* may be due to the reduced contact that the root of the former species has with the oil. Only the soluble fraction of the oil dissolves into the water column, with the insoluble portion forming a film on the water surface (Val and Almeida-Val, 1999). This implies that the insoluble part of the oil is not in contact with the root system of *E. crassipes*, a floating macrophyte. By contrast, both oil fractions (insoluble and soluble) penetrate the soil and can quickly reach the root system of an emergent macrophyte fixed to the substrate, such as *E. polystachya* (Lopes and Piedade, 2011).

We found that exposure period was another important factor in determining the effect of Urucu crude oil on *E. crassipes*. At the dosage of 0.5 L.m⁻² the plant began to die only after 35 days of exposure, but most of the biomass remained alive. At a dosage of 3.0 L.m⁻², toxic effects were recorded within 21 days, and at the end of the experiment all the live biomass had disappeared. The length of exposure was also important in determining the effects of Urucu crude oil on *E. polystachya*, with a reduction in leaf size occurring after only 20 days of exposure (Lopes and Piedade, 2009). *P. stratiotes* showed mortality after seven days at dosages of 0.1 and 0.2 L.m⁻² (Silva and Camargo, 2007). Therefore, both the concentration and the period of exposure

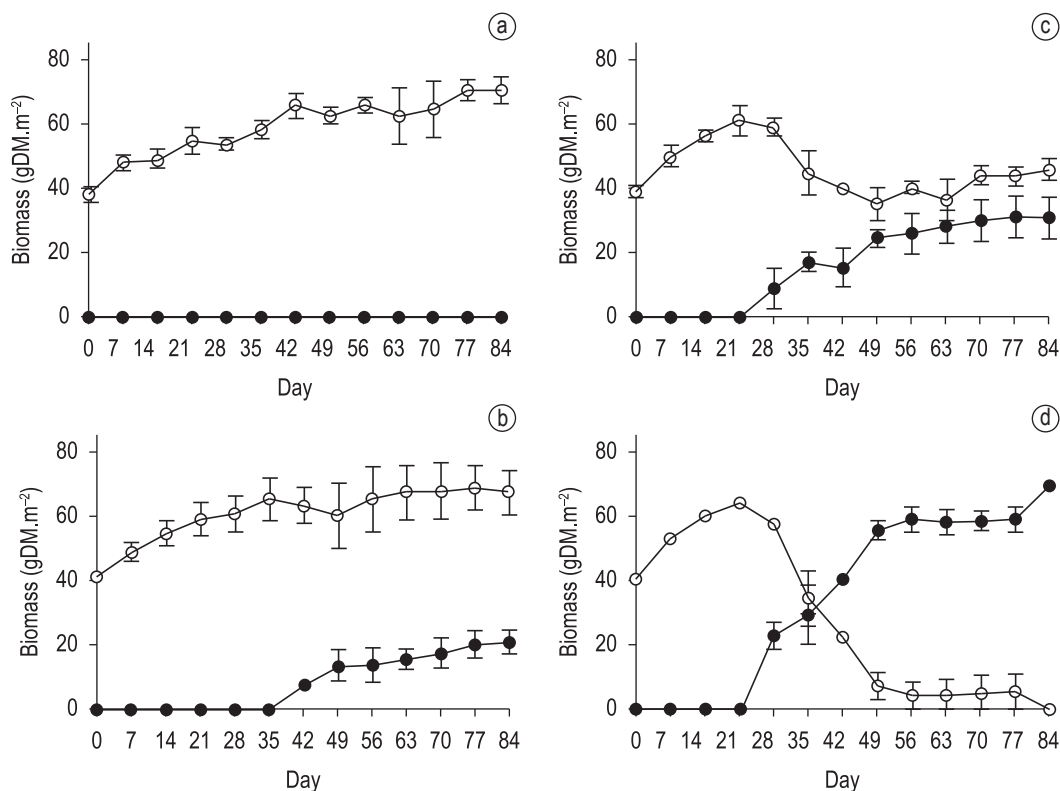


Figure 2. Means and standard deviations of live (○) and dead (●) biomass of *Eichhornia crassipes* in the four oil addition treatments: 0 (a); 0.5 (b); 1.5 (c) and 3.0 L.m⁻² (d).

to petroleum determine the intensity of the impact on *E. crassipes* and other aquatic macrophytes.

In addition to the effect of oil on this aquatic macrophyte, we observed changes in some abiotic characteristics of the water, especially in the treatment with the highest volume of oil. The decomposition of the oil and the dead biomass of *E. crassipes* reduced the dissolved oxygen and pH, and increased electrical conductivity and concentrations of total phosphorus in the water. A small reduction in oxygen concentration was also observed by Silva and Camargo (2007). The intensity of changes in the characteristics of the water is influenced by several factors, including the concentrations of oil and dead plant biomass. In this study, the amounts of oil used and the dead biomass of the plant were larger than in the study of Silva and Camargo (2007). However, despite the intensity, these changes may have consequences for other organisms than the aquatic macrophytes.

We conclude that an oil spill can cause total mortality in the local population of one macrophyte species, but not of another. This may alter the macrophyte species diversity in the impacted region. In the case of *E. crassipes* and *P. stratiotes*, an Urucu crude oil spill could favor *E. crassipes*, the less sensitive species.

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