Effect of Urucu crude oil on the aquatic macrophyte Pistia stratiotes.

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ABSTRACT: Effect of Urucu crude oil on the aquatic macrophyte Pistia stratiotes. We evaluated the influence of Urucu crude oil in three different concentrations (0.1 Lm^{-2}; 0.2 Lm^{-2} and 0.3 Lm^{-2}) on the aquatic macrophyte Pistia stratiotes. During a period of 14 weeks we measured the root length, rosette diameter, number of individuals, live and dead biomass, and the temperature, pH, electrical conductivity, turbidity and dissolved oxygen. The limnological variables did not significantly increase or decrease during the study period. Values of live biomass in the treatment without oil (control) were statistically higher than in the treatments with Urucu crude oil from the second week onwards. During weeks 1-4, dead biomass in the treatments with Urucu crude oil was significantly higher than in the control. All individuals in the control tanks remained alive, whereas the number of individuals in the treatments with Urucu crude oil decreased significantly.
Key-words: petroleum, live biomass, dead biomass, aquatic vegetation.

RESUMO: Efeito do petróleo de Urucu sobre a macrófita aquática Pistia stratiotes. O objetivo deste estudo foi avaliar a influência de três concentrações de petróleo de Urucu (0,1 Lm^{-2}; 0,2 Lm^{-2} e 0,3 Lm^{-2}) na macrófita Pistia stratiotes. Durante 14 semanas obtivemos valores de temperatura, pH, condutividade elétrica, turbidez, oxigênio dissolvido, comprimento da raiz, diâmetro de roseta, número de indivíduos e biomassa viva e morta. As variáveis limnológicas não variaram estatisticamente entre os tratamentos no período de estudo. Os valores de biomassa viva no tratamento sem petróleo de Urucu foram estatisticamente maiores a partir da segunda semana em relação aos tratamentos com petróleo. Durante as semanas 1, 2, 3 e 4, os valores de biomassa morta no tratamento com petróleo foram significativamente maiores do que o tratamento sem petróleo. Os indivíduos permaneceram vivos no tratamento sem petróleo. O número de indivíduos nos tratamentos com petróleo de Urucu reduziu-se significativamente.
Palavras-chave: petróleo, biomassa viva, biomassa morta, vegetação aquática.

Introduction

Aquatic macrophytes are frequently found in floodplain areas, especially in tropical regions, because of the favorable geological, hydrological and climate conditions (Esteves, 1988). Pistia stratiotes is a floating aquatic macrophyte that is originally from tropical America and is widely distributed in aquatic environments in Brazil, occurring in the Pantanal of Mato Grosso (Pott & Pott, 1994), artificially eutrophic reservoirs (Palombo, 1997; Lopes-Ferreira, 2000), coastal rivers of the state of São Paulo (Henry-Silva et al., 2001) and in the Amazon basin. Studies of the herbaceous vegetation (terrestrial and aquatic) in Amazonian floodplains have shown that 4 of the 17 most dominant species are aquatic macrophytes, including Pistia stratiotes (Junk & Piedade, 1997).

The Amazon basin is an important oil-producing region in Brazil. The oil is extracted from wells located in the vicinity of the Urucu River, and is used to produce derivatives of high aggregate value, such as diesel, naphtha and gasoline. The Urucu oil-bearing province produces a mean of 56.5 thousand barrels of petroleum per day, and is the second-largest producer on land in the country (Petrobras, 2007). With the increasing production of petroleum in the Amazon region, the risk of occasional accidental spills of oil or its derivatives has increased, and therefore the possibility of impact from oil on the biota of the aquatic ecosystems is higher.

The effect of oil on aquatic vegetation varies with the amount and kind of oil, which is classified in five categories: very light oil, such as gasoline; light oil, such as diesel, number 2 fuel oil, and light crude oil; medium light oils, such as the majority of crude oils; heavy oil, such as number 6 fuel oil; and very heavy oil, which does not float on water. This classification is imprecise, but can be used to indicate the effects of the different kinds of oil on the aquatic biota (Pezeshki et al., 2000). The Urucu crude oil is classified as light oil, because it produces mainly diesel. This kind of oil is the most toxic for plants and other organisms, compared to heavy oils (Pezeshki et al., 2000). Petroleum causes changes in the food chain, because it affects the primary producers (DeLaune et al., 1979) and consequently the aquatic fauna that uses the biomass of aquatic macrophytes.

The effects of oil on the vegetation of coastal brackish waters have been much studied. The studies have examined different factors, such as the kind and amount of oil spilled into the medium (Pezeshki et al., 2001; Lindau et al., 1999); the sensitivity and age of the plant species involved (Pezeshki et al., 2000); the direction of movement of the ocean waves, and consequently of the petroleum; and the growth stage of the plants, among others. Studies on the chemical toxicity and physical effects of petroleum on estuarine plants have also been carried out because of the intense activity (refining, storage and transport) in these areas and accidents with oil tankers such as the Torrey Canyon in 1967, the Exxon Valdez in 1989, the Erika in 1999, the Jessica in 2001 and the Prestige in 2002 (Laws, 2000; Born et al., 2003; Cadiou et al., 2004; Medina-Bellver et al., 2005).

DeLaune et al. (1979) and Pezeshki et al. (2000) observed that petroleum contamination of plants can delay seed germination, reduce their size, biomass or photosynthesis rate, or even cause complete mortality. The metabolism of estuarine plants can also be rapidly affected by oil spills, reducing gas exchange, transpiration and photosynthesis as the oil covers the stems and leaves (Lindau et al., 2003). However, there have been few studies of the effect of petroleum on freshwater aquatic macrophytes, and studies comparing the responses of different groups of aquatic plants are only in the initial stages (DeLaune et al., 2003). Therefore, it is fundamentally important to assess the levels of sensitivity of the different species of aquatic vegetation to petroleum, in order to predict its effects on both the plants and the aquatic ecosystem. The main objective of the present study was to evaluate the influence of different concentrations of Urucu crude oil on the growth of the floating aquatic macrophyte P. stratiotes, and to improve knowledge of the effects of this substance on aquatic plants.

Materials and methods

The fully randomized experiment was conducted during a 14-week period (January through April 2004) in a greenhouse (95% transparence) in the Experimental Garden of the Biosciences Institute, UNESP, Rio Claro Campus in the state of São Paulo, Brazil. Twelve experimental tanks (0.19 m^2) were set up, with equal quantities of water and plants, totalling 4 treatments in triplicate with different concentrations of Urucu crude oil. The treatments were: (1) control, without oil; (2) 0.1 Lm^{-2}; (3) 0.2 Lm^{-2} and (4) 0.3 Lm^{-2} of oil. Water lost through evapotranspiration was replaced weekly with water from the collection locality.

The root length and rosette width of each individual were measured weekly, and the (fresh) live and dead biomasses in each experimental tank were weighed after the excess water was allowed to drain from the plants for 5 minutes, according to Agami & Reddy (1990). The temperature, pH, electrical condutivity, turbidity and dissolved oxygen were measured weekly with a Horida U-10 multi-sensor.

The dry biomass of P. stratiotes was estimated from a linear regression between fresh biomass and dry biomass of individuals collected on the south coast of São Paulo:

$DB = -0.1663 + (0.0624)\ FB$ ($r^2 = 0.9864$), where DB = dry biomass, FB = fresh biomass.

The analysis of variance (one-way ANOVA), with a 5% confidence interval, was applied to the results for root length, rosette diameter, number of individuals, live and dead biomass to test for significant differences between the treatments in each week. Subsequently a Tukey's test was used to identify differences among treatments (Zar, 1999).

Results and discussion

The limnological variables (Tab. I) did not differ among the four treatments.

Table I: Means and standard deviations (14 weeks) of the physical and chemical variables of water in the different treatments.

Variable \ Treatment	1	2	3	4
pH	7.5 ± 0.59	7.57 ± 1.03	7.55 ± 1.13	7.40 ± 1.19
Conductivity (mScm^{-1})	0.049 ± 0.014	0.081 ± 0.027	0.071 ± 0.013	0.066 ± 0.013
Turbidity (NTU)	2.02 ± 2.10	4.26 ± 4.28	4.69 ± 2.86	3.36 ± 2.81
Temperature (°C)	23.89 ± 3.43	24.6 ± 3.89	24.85 ± 3.99	24.96 ± 3.98
Dissolved oxygen (mgL^{-1})	6.00 ± 1.03	5.62 ± 0.89	5.19 ± 0.93	5.00 ± 1.16

Probably the low concentrations of oil used in this study were not sufficient to cause differences in the physical and chemical variables of the water. However Crema (2003), studying the influence of Urucu oil on the floating aquatic macrophyte Eichhornia crassipes, observed significant differences in dissolved oxygen and electrical conductivity of the water between the treatments, and attributed these differences to decomposition of the petroleum and the plant biomass. We note the author used concentrations of oil up to ten times greater (3 Lm^{-2}) than in the present study. In our experiment, we observed the presence of algae in all the treatments with oil, which may also explain why the oxygen concentrations remained steady, even in the higher oil concentrations.

The amounts of living biomass in the control treatment were significantly higher ($P<0.001$, in the 14th week) than in the other treatments beginning with the second week, whereas the amounts of dead biomass were significantly higher ($P<0.05$, in the 6th week) than the control in the treatments with oil during the first six weeks. Fig. 1 illustrates the reduction of approximately 50% of living biomass in Treatment 4 in the second week.

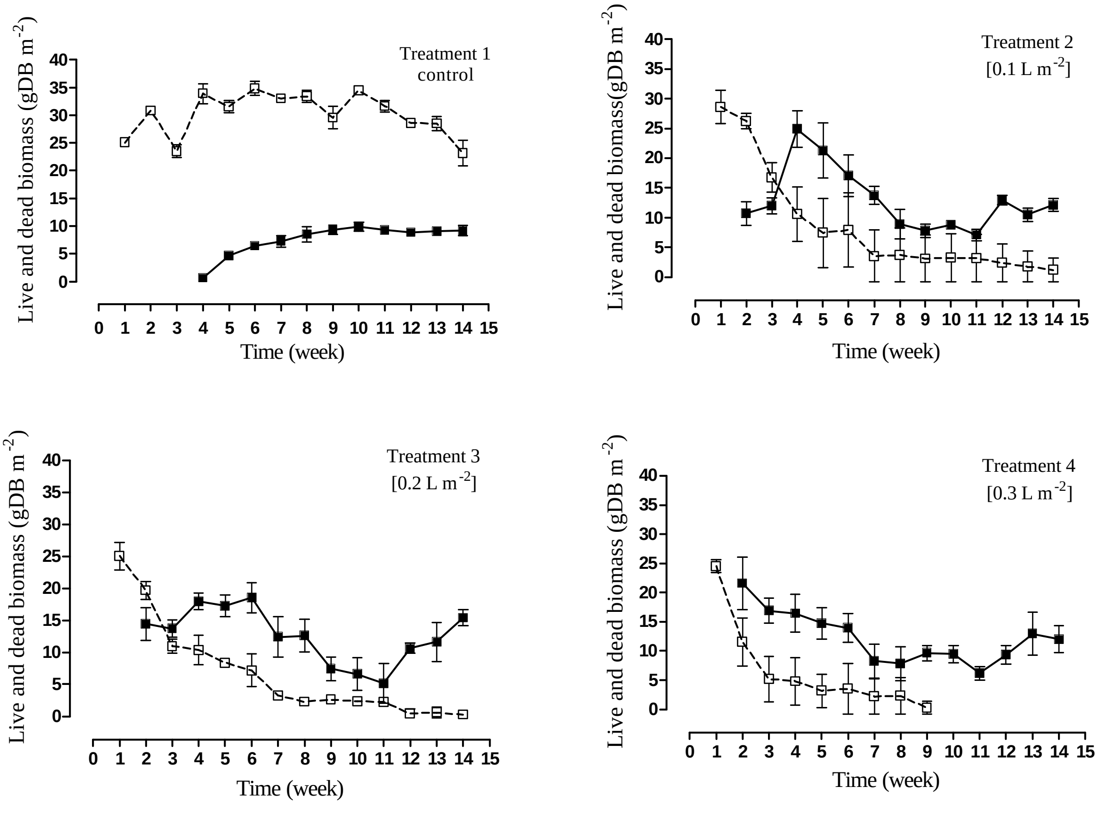

Figure 1: Means and standard deviations of living (□) and dead (■) biomass of P. stratiotes in the different treatments.

In the control treatment, dead biomass was observed only from the fourth week on, whereas in the treatments with oil, dead biomass was observed beginning in the second week. These results demonstrated that P. stratiotes is highly sensitive to Urucu crude oil: only 0.1 Lm^{-2} oil was sufficient to reduce growth and cause mortality to the plant. This sensitivity is more evident compared to the results obtained by Crema (2003), who studied the effect of Urucu crude oil on E. crassipes. The author observed growth of E. crassipes at concentrations of 1.5 and 3.0 Lm^{-2} Urucu crude oil until the fifth week, and only after this period observed the appearance of dead biomass. Pezeshki et al. (2001) observed a reduction in the aerial biomass of the emergent macrophytes Spartina patens and Sagittaria lancifolia, following application of 2 Lm^{-2} of South Louisiana crude oil to the leaves.

The high sensitivity of P. stratiotes to Urucu crude oil is probably related to the characteristics of its leaf blade, which was described by Pott & Pott (2000) as spongy, and by Lorenzi & Souza (1995) as velvety. When a plant of P. stratiotes comes into contact with Urucu crude oil, its leaves act as absorbent paper and the entire leaf surface comes to be covered with a thin layer of oil. According to Pezeshki et al. (2000), oil can affect plants both physically and chemically, but in the case of P. stratiotes the physical effect is probably more important. Pezeshki & DeLaune (1993) observed that the oil covers the leaf surface and blocks the stomates, thus limiting the entry of carbon dioxide, reducing photosynthesis and limiting plant growth. The oil, besides causing oxygen stress in tissue growth because of the reduction of gas exchange, also ruptures the root membranes (DeLaune et al., 2003).

The results indicated that application of Urucu crude oil caused a significant reduction in the number of individuals of P. stratiotes ($P<0.001$, in the 14th week). Fig. 2 shows that the number of individuals in Treatment 1 (control) remained unchanged during the 14 weeks of the experiment; however in the treatments with oil, there was a significant decline in the number of individuals. In Treatment 4, all the plants died in the 14th week. DeLaune et al. (2003) also observed a significant reduction of individuals of Spartina patens after they were exposed to 2 Lm^{-2} of South Louisiana crude oil.

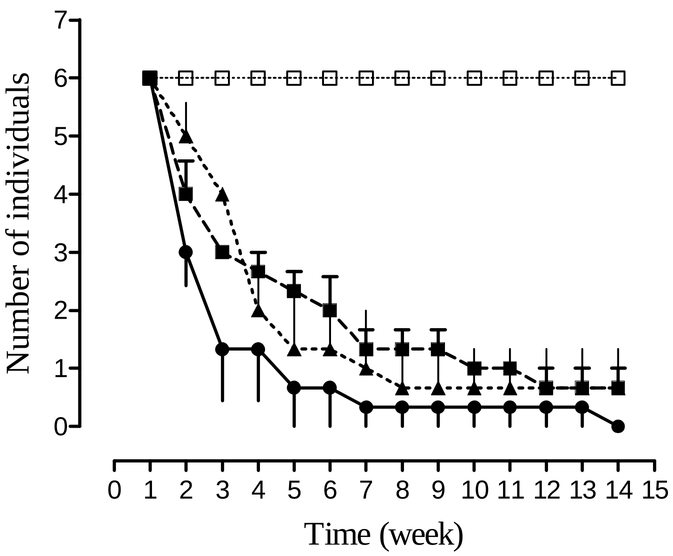

Figure 2: Means and standard deviations of the number of individuals of P. stratiotes in Treatments 1- control (□), 2- 0.1 Lm^{-2} (▲); 3- 0.2 Lm^{-2} (■) and 4- 0.3 $L.m^{-2}$ (●).

Changes in the root length and the rosette diameter were observed in all treatments, including the control. In the treatment without petroleum, the root increased in length and the diameter of the rosette decreased slightly. However, the amounts of root length and the rosette diameter in the control treatment were significantly higher then in the other treatments ($P<0.005$, in the 14th week).

The reduction in rosette diameter in the control treatment indicates that probably the experimental conditions were not favorable to the growth of this species. Although the tanks were filled with water from an environment where the species shows intense growth (Rubim, 2004), probably the lack of renewal of the water limited the growth of the species. Xie & Yu (2003) observed an increase in the root length of E. crassipes in nutrient-poor water. On the other hand, in the treatments with oil, both the root length and the rosette diameter decreased (Fig. 3 and 4).

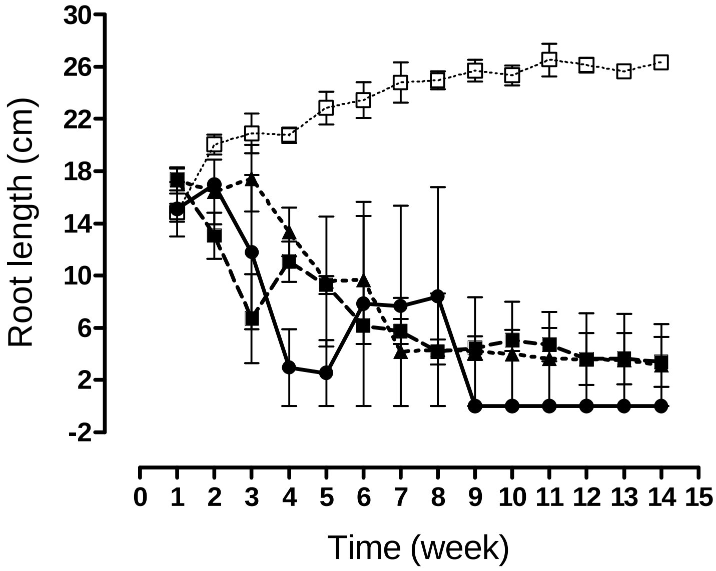

Figure 3: Means and standard deviations of root length in Treatments 1- control (□), 2- 0.1 $L.m^{-2}$ (▲); 3- 0.2 $L.m^{-2}$ (■) and 4- 0.3 $L.m^{-2}$ (●).

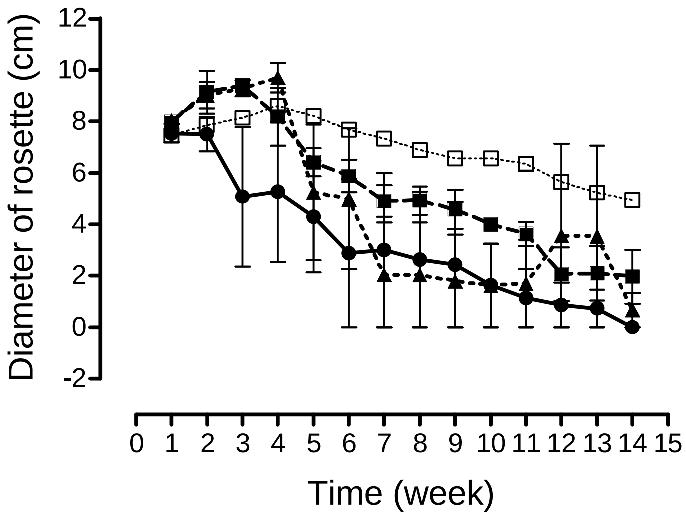

Figure 4: Means and standard deviations of the rosette diameter in Treatments 1- control (□), 2- 0.1 Lm^{-2} (▲); 3- 0.2 Lm^{-2} (■) and 4- 0.3 Lm^{-2} (●).

These results illustrate the damaging effect of the oil on this species of aquatic macrophyte.

The sensitivity of P. stratiotes and of the different ecological types of aquatic macrophytes is apparent from the observations presented in Tab. II. Higher concentrations of oil were used in the studies with species of emergent aquatic macrophytes than with floating species, and there was regeneration of individual plants or parts of plants in most of the studies with emergent species. The resistance of S. alterniflora to oil is higher when its leaves are not touched by the oil (DeLaune et al., 1979). P. stratiotes is the most sensitive species, because its leaves inevitably come into contact with the oil, and are not immediately resistant to high oil concentrations.

Table II: Effects of different types of crude oil on aquatic macrophyte biomass. * leaves in contact with oil. **recovery (leaves, roots and/or individuals).

Concentration	Species(ecological type)	Effect on biomass	Reference
32 Lm^2 SLC	Spartina alterniflora** (emergent)	no significant difference	DeLaune et al. (1979)
8 Lm^2 SLC	Spartina patens (emergent)	significant reduction	Lin & Mendelssohn (1996)
2 Lm^2 SLC*	S. alterniflora**	significant reduction	Lindau et al. (1999)
2 Lm^2 SLC*	S. patens**	significant reduction	Pezeshki et al. (2001)
^{3}2.5 Lm^2 (soil surface) of No. 2 fuel oil	S. alterniflora	significant reduction	Lin et al. (2002)
1.5 and 3 Lm^2 Urucu oil*	Eichhornia crassipes (floating)	significant reduction	Crema (2003)
0.5 Lm^2 Urucu oil*	E. crassipes	no significant difference	Crema (2003)
2 Lm^2 SLC*	S. alterniflora** and S. patens	significant reduction	DeLaune et al. (2003)
2 Lm^2 SLC*	S. alterniflora** and S. lancifolia**	25% increase in biomass production	Lindau et al. (2003)
0.1; 0.2 and 0.3 Lm^2 Urucu oil*	Pistia stratiotes (floating)	significant reduction	present study

Comparing the tolerance to oil of the two floating aquatic macrophytes P. stratiotes and Eichhornia crassipes, it can be concluded that an oil spill in an area colonized by both species could cause mortality of P. stratiotes. Because E. crassipes is more resistant to oil, this species could increase its biomass and area occupied, because the elimination of P. stratiotes would reduce competition pressure, benefiting the growth of E. crassipes. The effect of oil on plants depends on several factors, including the concentration of the oil, the ecological type of the plant, and amount of the contact of the leaves with the oil.

It is concluded that the aquatic macrophyte Pistia stratiotes is very sensitive to oil, because the oil killed all the individual plants within 14 weeks at a concentration of 0.3 Lm^{-2}. Pistia stratiotes is much more sensitive than the floating species Eichhornia crassipes, and also than emergent species, because much higher concentrations of petroleum (10 times higher in the case of E. crassipes) are necessary to cause mortality in these species.

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