

# Effects of crude oil on survival, morphology, and anatomy of two aquatic macrophytes from the Amazon floodplains

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Received: 4 March 2009 / Revised: 23 September 2009 / Accepted: 28 September 2009 / Published online: 19 October 2009  
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**Abstract** Aquatic herbaceous macrophytes grow in profusion in the Amazon fertile varzea floodplains. A large number of species occur but only a few are particularly abundant, supporting food chains, contributing substantially to carbon and nutrient cycles. Their growth and role in the ecosystem depend, among other, on its life cycles and habits, floating or semi-aquatic. Although in the last decades, petrolierous activity intensified in the Central Amazon region and so did oil spills, the effect of petroleum on the native aquatic plants is unknown. The present study was designed to test experimentally the survival and morpho-anatomical modifications of the free floating water hyacinth *Eichhornia crassipes* and the semi-aquatic grass *Echinochloa polystachya* to 10 different concentrations of crude oil. Higher concentrations of crude oil caused the mortality in both species; however, lethal dose ( $LD_{50}$ ) values showed that *E. polystachya* was more sensitive than

*E. crassipes*. Despite the higher tolerance of *E. crassipes*, the inhibition of root and leaf growth as well as anatomical modifications in leaves were registered in higher concentrations. Additionally, the oil caused a reduction in leaf numbers in both species. Although mortality of the floating species was lower, it may increases over time, since important alterations in morphology and anatomy occurred. These results show that oil spills in the Amazon varzea can cause severe alterations in the aquatic flora and in the floodplain dynamics.

**Keywords** Herbaceous plants · *Echinochloa polystachya* · *Eichhornia crassipes* · Central Amazon · Bioindicators

## Introduction

Petrolierous regions are under the constant risk of accidents, as much during the process of extraction, as during the transport of crude oil (Val & Almeida-Val, 1999; Keramitsoglou et al., 2003). In the Amazon, the oil extracted in Urucu (Coari, Amazonas State-AM, Brazil) is transported by rafts along the Solimões River, to the refinery in Manaus, AM. An oil spill accident during such transportation could seriously affect the floodplain (Val & Almeida-Val, 1999), particularly the low varzea areas (Wittmann et al., 2002) where a greater density of aquatic grasses occurs (Junk & Piedade, 1993).

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Handling editor: S. M. Thomaz

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Amazonian aquatic grasses are very important for the aquatic ecosystem because of their huge biomass accumulation. Some species, such as *Echinochloa polystachya* (H.B.K.) Hitchcock, Poaceae, can produce 108 t ha<sup>-1</sup> year<sup>-1</sup> of dry matter (Piedade et al., 1991). Moreover, its submerged phytomass forms a complex environment, composed of stems and roots that are colonized by invertebrates and algae (Junk, 1973), in which many species of fish are reliant (Junk & Howard-Williams, 1984; Sanchez-Botero & Araujo-Lima, 2001, Claro-Jr. et al., 2004). Although petroleum extraction has taken place in the central Amazon region for more than a decade, studies concerning crude oil toxicity in these plants are rare (Lopes, 2007).

The impact of crude oil on the environment depends on the specific characteristics of both the crude oil and the environment. Light oils are easily absorbed and, since they act at the cellular level, are immediately toxic to the plants. Heavy oils cover the plants causing asphyxia and hindering gaseous exchange (Pezeshki et al., 2000). When oil spills occur in a terrestrial environment, it directly affects the ecosystem through soil contamination; however, in aquatic environments, the floating oil on the water surface is dispersed through the action of wind and waves in the littoral region, thus affecting the terrestrial environment and vegetation (Pezeshki et al., 2000). In the case of the Amazon region, this dispersion might be maximized by the oscillation of the water level, due to the flooding pulse (Junk et al., 1989).

The entrance of petroleum into the aquatic-terrestrial system results in a series of alterations, such as the substantial increase of organic material as a result of the death and decomposition of plants (Akinluyi & Odeyemi, 1987), alterations in pH of the soil and the introduction of heavy metals, such as nickel and mercury (Ekundayo & Obuekwe, 2000). These alterations, coupled with the differentiated sensitivity of each species of plant, result in modifications in the composition of the community of aquatic plants (Burk, 1977; Pezeshki et al., 2000).

The difference in sensitivity among aquatic plants allows the use of several species to detect contamination (Swanson et al., 1991; Lewis & Wang, 1997; Mohan & Hosetti, 1999). The free-floating *Eichhornia crassipes* (Mart.) Solms. (water hyacinth), Pontederiaceae, and the emergent *Echinochloa polystachya* are two of the most important herbaceous species

colonizing the white-water varzea floodplains in the Amazon. Due to its high tolerance to many pollutants, *E. crassipes* is one of the most studied floating aquatic plants, being a promising species for decontamination of polluted areas since the species has high capacity to absorb and tolerate elevated levels of heavy metal ions (Harley, 1990). On the other hand, rooted plants like *E. polystachya* are often more sensitive to contaminants than the floating macrophytes, and commonly used as biomonitoring (Lovett-Doust et al., 1994; Lewis, 1995; Roshon et al., 1999). Therefore, evaluating the oil toxicity of these plant species with different habits may be a good way to estimate the oil impact on the ecosystem (Lewis, 1995).

Considering the potential risk of accidents due to petroliferous activity and the importance of these two plants for the ecosystem this study aims to: (1) determine the median lethal concentrations for species *E. crassipes* and *E. polystachya* exposed to the Urucu crude oil; (2) evaluate changes in leaf production, and leaf and root length for both species; and (3) perform an anatomical analysis of leaves of *E. crassipes* with visible modifications.

## Materials and methods

The aquatic macrophytes were collected on Marchantaria island (03.15S, 060.00W), in the Central Amazon, Amazonas, Brazil in May of 2006. The two species used have been chosen due to their wide distribution throughout the floodplain, for being native to the Amazon and typical of the varzea ecosystem. The soil for the experiment with *E. polystachya* was sampled in the same location. The experiment was performed in the greenhouse of the project INPA/Max-Planck, Manaus, Amazonas, Brazil.

Crude oil from the Petroliferous Base of Urucu (Amazonas, Brazil) was used in the experiment. This oil is considered light, with 45.8 API degrees (ANP, 2008). See Table 1 for oil composition (Petrobrás, 1997).

## Experimental design

A total of 130 young *E. crassipes* plants, with three leaves of ~4 × 7 cm, were washed in tap water in the laboratory, for the removal of sediment and placed in vases with 2 l of water where they remained for 30 days for growth and acclimatization.

**Table 1** Composition of Urucu crude oil (Petrobrás, 1997)

Motor octane number (distillation cut point 16–180°C)	38.0
Hydrocarbon types (% of total; distillation cut point 16–180°C)	
Paraffins	64.8
Naphthalenes	20.2
Aromatics	15.0
Hydrocarbon types (% of total; distillation cut point 144–244°C)	
Saturates	85.7
Aromatics	13.2
Olefins	1.1

Whenever necessary, the water level was adjusted. Parts of the culm of mature plants of *E. polystachya* were collected, and from each one, a 15-cm part containing one node was taken. The propagules were planted in vases with 2 l of varzea soil (70.5% clay, 17.92% silt, 8.97% coarse sand, 2.61% fine sand) (A. Lopes, unpublished data). After 30 days, 130 young plants with at least 30 cm of height were selected to perform the experiment. The plants were irrigated daily throughout the experimental period using water from an artesian well. Sample units consisted of the 260 plastic vases (20 cm of diameter by 18.5 cm of height) distributed in factorial experiments in a completely randomized design 2 × 10 (species × oil dosage) with 13 replicates per treatment.

After the period of acclimatization and growth, crude oil was placed on the surface of the soil and water without mixing with the substratum and avoiding direct contact with leaves. Plants were randomly distributed in 10 treatments (0, 0.08, 0.16, 0.32, 0.66, 1.32, 2.64, 5.29, 10.59, and 15.89 l m<sup>-2</sup>). Leaves with more than 30% of green area were counted weekly, and their length and width were measured over 35 days (*E. polystachya*) and 90 days (*E. crassipes*). The root length was measured in the beginning and at the end of the experiment for *E. crassipes*. This measurement was not possible in *E. polystachya* which was fixed in the ground. In order to calculate the value of the lethal dose (LD<sub>50</sub>), the plants remained in the treatments for 132 days with the same oil dosages to compare the time of survival of both species. For assessment of the LD<sub>50</sub>, the OECD toxicity protocol 227 was used (OECD, 2006).

The anatomical analyses of *E. crassipes* leaves were carried out after 90 days of exposure to crude oil,

by sampling leaves presenting visible alterations and leaves in the control group and storing them in 70% ethanol. Transversal sections were performed in the bulb and the median region of leaves for the mounting of semi-permanent microscope slides. The histological sections were cleared with sodium hypochlorite, washed with distilled water, stained with Safra-blau coloration, placed over microscope slides and cover slips with a drop of glycerin, and embedded with nail polish. Observations of the histological sections were performed using an optic microscope Leica<sup>TM</sup>, in 5× to 40× objectives. Photomicrographs were taken using an optic microscope Zeiss<sup>TM</sup> MC 63 in objectives 2.5×, 20×, and 40×.

### Statistical analysis

Statistical analyses were performed using the following software: Systat 10.2 (Systat, 2002); Microsoft Office Excel 11.0 (Microsoft Corporation, 2003); and Trimmed Spearman–Karber Method (Hamilton et al., 1977). In order to evaluate the effect of the time and the dosage of crude oil on leaf length and the increase in leaf number, we used two-way ANOVA for repeated measures taking into account assumptions of homocedasticity and sphericity. Data from day 0 to the 35th day of plant exposure to crude oil were used to analyze the effect of crude oil on leaf length. This was done to minimize the amount of sample reduction due to mortality in treatments of a higher concentration. Furthermore, for *E. polystachya* the data for concentrations up to 0.32 l m<sup>-2</sup> were used, due to the high mortality of the plants from the beginning of the experiment. The difference between the root length of *E. crassipes* in the beginning and at the end of experiment was compared by two-way ANOVA. In order to confirm the result, a paired *t* test for each group of treatment was used. An estimation of LD<sub>50</sub> values with a dosage able to kill 50% of the tested population was obtained through the statistic method of Spearman–Karber, with 95% of confidence interval (Hamilton et al., 1977; OECD, 2006).

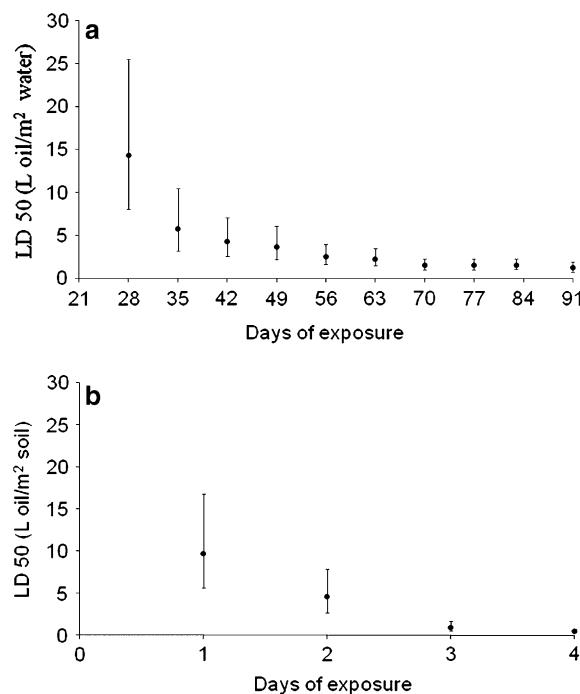
### Results

Seventy days after the application of crude oil, the death of all the plants of *E. crassipes* exposed to the concentrations of 10.59 and 15.89 l m<sup>-2</sup> was

observed. *E. polystachya* was even more sensitive to crude oil as 4 days after the exposure all plants exposed to the 5.29, 10.59, and 15.89 l m<sup>-2</sup> died and after 35 days all plants exposed to dosages above 0.66 l m<sup>-2</sup> also died. In *E. crassipes*, many modifications in the plants exposed to crude oil were observed. These modifications include the reduction of total plant size, shortening of bulbs, and reduction of size and thickness of leaves.

At 91 days of exposure, the LD<sub>50</sub> for *E. crassipes* was calculated as 1.24 l m<sup>-2</sup> (Fig. 1a). For *E. polystachya*, after 4 days the LD<sub>50</sub> was 0.47 l m<sup>-2</sup> (Fig. 1b). After 49 days, the *E. polystachya* had reached 100% of mortality in all treatments, except the control, which did not show growth inhibition.

The ANOVA with repeated measures showed that leaf increase of *E. crassipes* (Fig. 2a) throughout 35 days was affected by crude oil dosage ( $F(9, 120) = 9.16, P < 0.0001$ ), by exposure time ( $F(5, 600) = 18.72, P < 0.0001$ ), and by the interaction between dosage and exposure time ( $F(45, 600) = 6.51, P < 0.0001$ ). During the same period of time, however, with only a 0.32-l dosage, *E. polystachya* (Fig. 2b) was affected by dosage ( $F(3, 21) = 4.013$ ,



**Fig. 1** Toxicity of Urucu's crude oil expressed in DL<sub>50</sub> values, for: **a** *E. crassipes*; **b** *E. polystachya* throughout exposure time. Values with 95% confidence intervals

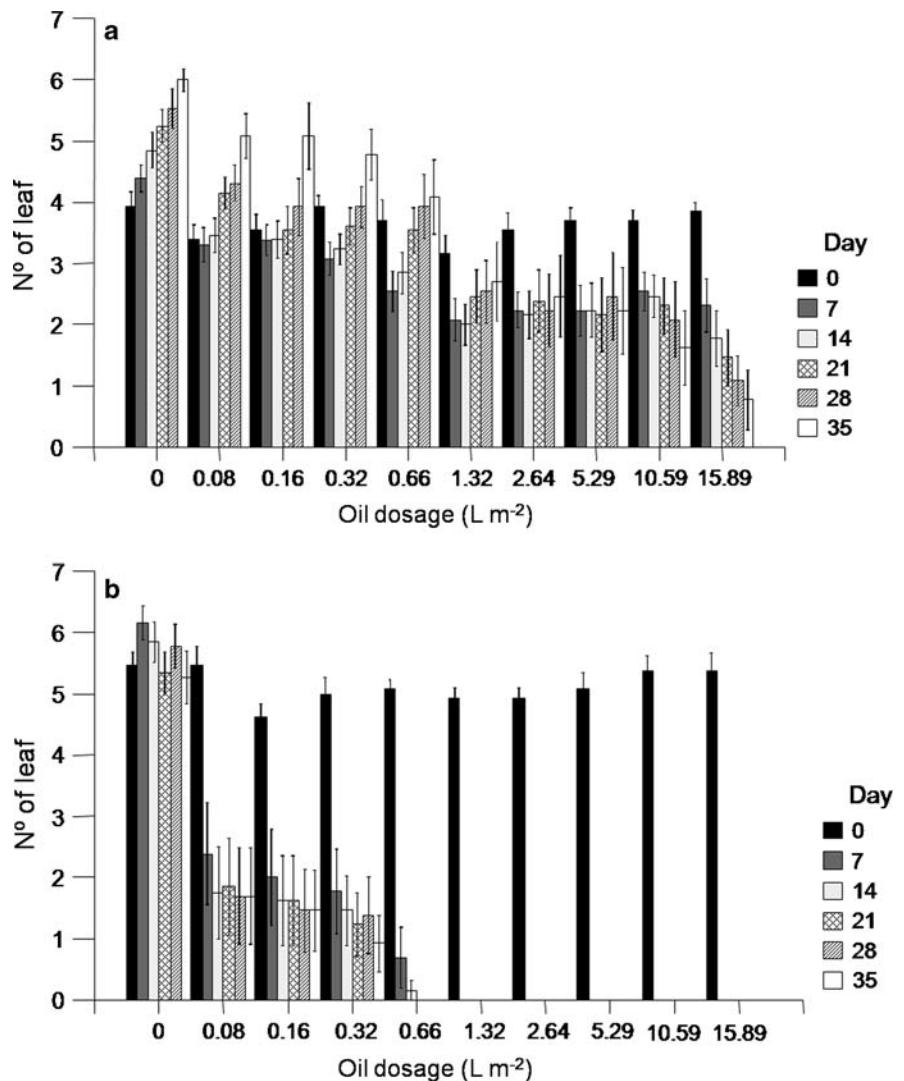
$P = 0.021$ ) and exposure time ( $F(5, 150) = 2.972, P = 0.015$ ), the interaction between these factors was not significant ( $F(45, 150) = 1.583, P = 0.091$ ).

Length and width of *E. crassipes* leaves showed a strong correlation ( $r = 0.89$ ), therefore only the results for length are presented. There was a reduction of leaves in *E. crassipes* due to the dosage of crude oil ( $F(9, 119) = 2.84, P = 0.005$ ) and to exposure time ( $F(5, 595) = 48.22, P < 0.0001$ ), there was a significant interaction between these factors ( $F(45, 595) = 4.10, P < 0.0001$ ). In Fig. 3a, the stabilization pattern of leaves in treatments ranging from 0 to 0.08 l is very similar, while there is a pronounced growth reduction in concentrations greater than 1.32 l m<sup>-2</sup>. There was no significant effect of crude oil dosage on the leaf length of *E. polystachya* ( $F(3, 21) = 1.48, P = 0.249$ ), although exposure time presented a significant effect ( $F(5, 105) = 26.469, P < 0.0001$ ). As Fig. 3b shows, there was no significant interaction between crude oil dosage and exposure time ( $F(45, 105) = 1.391, P = 0.165$ ).

The difference in the *E. crassipes* root length between the beginning and the end of the experiment was influenced by the dosage of oil ( $F(9, 120) = 2.27, P = 0.02$ ). Individual differences were found between the treatments 0.08 and 5.29 l m<sup>-2</sup> ( $P < 0.05$ ). The paired *t* test confirmed that the oil dosage caused root growth inhibition, except in the 0.66 l m<sup>-2</sup> dose, where there was stimulation of growth, compared to the control group (Fig. 4). In the other dosages, there was no significant difference between initial and final root length, varying from 8 to 12 cm at the end of the experiment.

It was not possible to carry out anatomy analysis of *E. polystachya* leaves due to high mortality in all treatments. Anatomic analysis of *E. crassipes* leaves showed an increase in the size of aerenchyma air spaces in treatments 5.29 and 10.59 l m<sup>-2</sup> and in higher dosages this tissue disappeared (Fig. 5a–d). There was also a displacement of aerenchyma air spaces in treatments 5.29 and 10.59 l m<sup>-2</sup> (Fig. 5b, c), which were organized in the central region of the leaf in the control group (Fig. 5a). Palisade parenchyma was gradually diminished with crude oil dosage, until it could no longer be visualized in treatment 15.89 l m<sup>-2</sup> (Fig. 5d). In the control group (Fig. 5a), a vascular cylinder was observed in the central line of the leaf area but this suffered a drastic disorganization due to exposure to the oil (Fig. 5b–d). The main

**Fig. 2** Dosage effect of crude oil and exposure time (days) on the leaf number of: **a** *Eichhornia crassipes*; **b** *Echinochloa polystachya*. Mean values with standard error ( $n = 13$ )



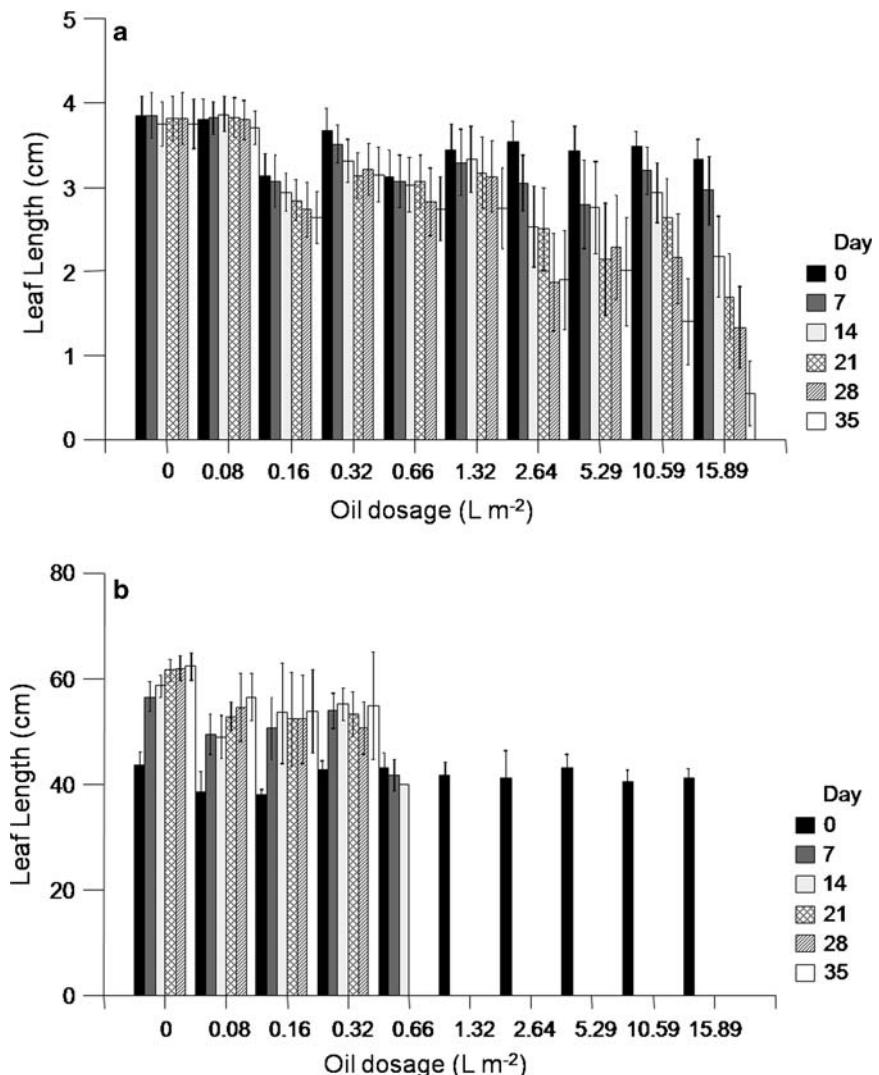
change observed in the bulb of *E. crassipes*, beyond the morphologic alterations such as the drastic reduction of the size and thickness of the bulb, was the reduction of the cellular size with the increase of oil dosage (Fig. 6a–d). Moreover, there was the disappearance of aerenchyma tissue in the treatments with higher dosages of crude oil (Fig. 6b–d).

## Discussion

The effect of petroleum hydrocarbons on the mortality of plants has been reported by several authors as an efficient measure of crude oil impact on the

affected ecosystem (Mendelsohn & McKee, 1988; Pezeshki et al., 2001; Lin et al., 2002). Besides the hydrocarbons that are toxic to plants, Urucu crude oil has 5 ppm of nickel, 5 ppm of vanadium, and 0.05% (wt) of sulfur (Petrobrás, 1997; ANP, 2008). The toxicity of heavy-metal ions is due chiefly to their interference with electron transport in respiration and photosynthesis, and the inactivation of vital enzymes. As a result, the energy status is lowered and the uptake of mineral nutrients decreases, reducing growth (Larcher, 1995). The magnitude of such an impact for the environment can be evaluated by means of alterations in the growth and development of the plants.

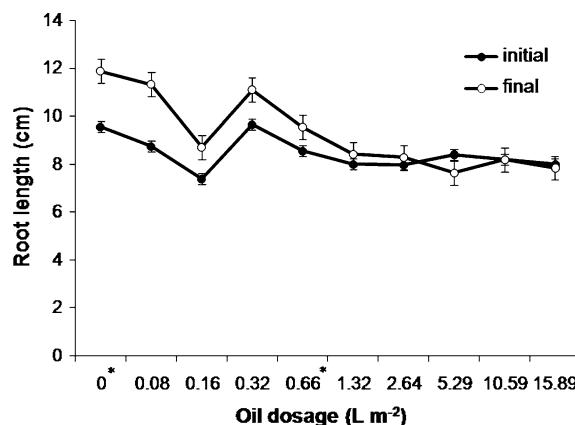
**Fig. 3** Effect of crude oil concentration and exposure time (days) on leaf length of: **a** *Eichhornia crassipes*; **b** *Echinochloa polystachya*. Means values with standard error ( $n = 13$ ). For *E. polystachya*, statistical analysis was performed at a concentration of  $0.66 \text{ L m}^{-2}$



Due to the high mortality of *E. polystachya*, it was not possible to verify the effect of crude oil on the growth of this species. This suggested that concentrations above  $0.32 \text{ L m}^{-2}$  may be limiting for this species. For *E. crassipes*, crude oil caused a reduction of leaf size and even though some individuals survived for a longer time, they presented morpho-anatomical modifications, irregular distribution of the aerenchyma air spaces or even its absence. The reduction of thickness of aerenchyma air spaces occurred in the bulb of plants exposed to dosages above  $0.66 \text{ L m}^{-2}$ . Aerenchyma is an adaptation in aquatic plants which gives buoyancy and allows the photosynthetic parts to be maintained above the water column (Junk & Piedade, 1997). The loss of this

adaptation in the leaves and in the bulb may cause the plant to sink increasing its contamination and resulting in its death.

Light oils such as the one found in Urucu act on the cellular level changing membrane permeability or interrupting several metabolism processes of the plant. Thus, their toxicity for the plants is immediate. On the other hand, the effect of heavy oils is gradual by causing physical damage, which results in asphyxia and the interruption of gas exchange (Pezeshki & DeLaune, 1993). In this study, morpho-anatomical modifications which suggest a drastic modification of plant metabolism were found. The reduction of leaf size and the loss of palisade parenchyma in *E. crassipes* are important factors in

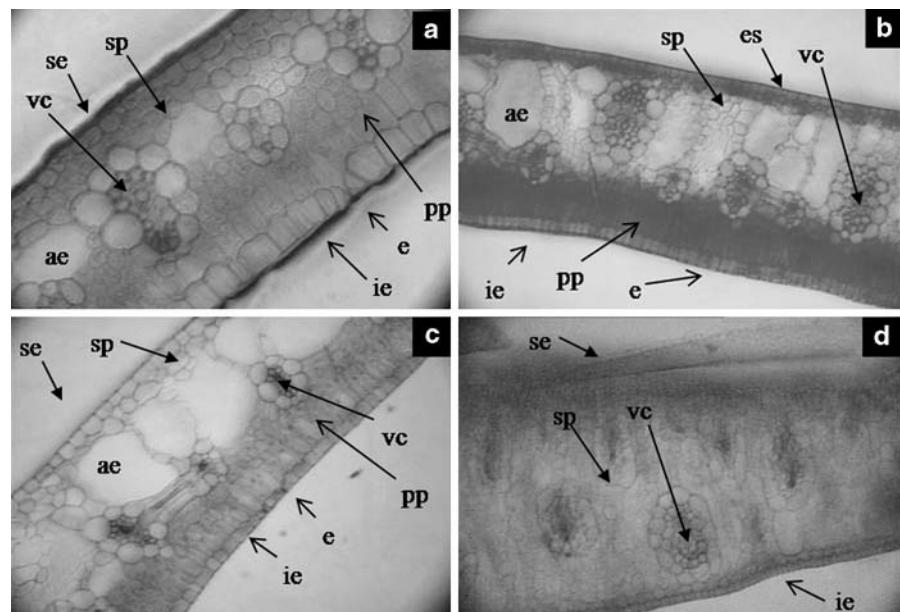


**Fig. 4** Effect of crude oil concentration on *E. crassipes* root length. Mean values with standard error ( $n = 13$ ). \* Indicates the treatments where there is a significant difference between the initial and final root length value ( $\alpha = 0.05$ )

the subsequent death of the plants. This indicates the high degree of stress caused by this pollutant.

The reduction of thickness and even the loss of palisade parenchyma tissue resulting from exposure to the oil indicate that, even if the plant produces new leaves they will have their functions compromised. Palisade parenchyma is located generally in the adaxial or upper surface of the leaves and consists of areas of elongated cells, rich in chloroplasts, placed perpendicularly to the surface of the foliar limb, presenting intercellular spaces (Souza, 2003).

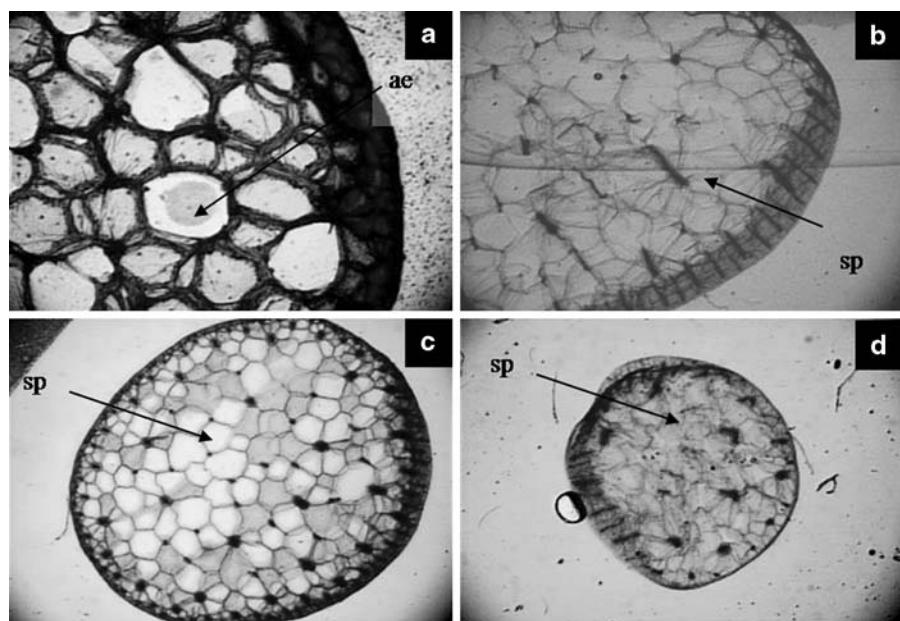
**Fig. 5** Transverse sections of *Eichhornia crassipes* leaves after 90 days of exposure to Urucu crude oil, at concentrations of: **a** 0; **b** 2.64; **c** 5.29; **d**  $15.89 \text{ L m}^{-2}$  of water. *ie* Inferior epidermis; *se* superior epidermis; *pp* palisade parenchyma; *sp* spongy parenchyma; *ae* aerenchyma air space; *e* estomate; *vc* vascular cylinder. Magnification of  $\times 400$  for control and  $\times 200$  for other treatment groups



This is the main tissue responsible for the photosynthesis of tracheophytes and presents high plasticity in response to environmental factors for a wide diversity of plants (Justo et al., 2005), including *E. crassipes*, as demonstrated in this study.

Based on experiments with industrial effluents, Ferreira & Graça (2002) concluded that radicle development is a good endpoint for toxicity tests with aquatic macrophytes. The inhibition of root elongation of *E. crassipes* occurred in doses of petroleum that may be considered too low, from 0.08 to  $15.89 \text{ L m}^{-2}$  of water. This confirms root elongation as a robust parameter that may be used to assess the damage caused by oil in this species. According to the study of Lin et al. (2002), an inhibitory effect on the length of roots of *Spartina alterniflora* Loisel. was found when using the oil No. 2 in dosages from 5 to  $20 \text{ L m}^{-2}$ . Studies on herbaceous plants suggest that root growth and physiological activity are dependent upon a continuous supply of assimilate (Richards, 1989). The inhibition of root growth causes a reduction in nutrient absorption, increasing the plant's stress and hampering their development. Disturbance in the absorption of nutrients leads to adjustments in distribution of assimilates, in the ratio of shoot to root growth, inducing, eventually, premature flowering and leaf abscission (Larcher, 1995). Although in the present study the roots of *E. polystachya* were not measured, Lopes & Piedade (2009) did not find changes in the

**Fig. 6** Transverse sections of *Eichhornia crassipes* bulb after 90 days of exposure to Urucu crude oil, in dosages of: **a** 0; **b** 2.64; **c** 5.29; **d** 15.89 l m<sup>-2</sup> of water. *sp* Spongy parenchyma; *ae* aerenchyma air space. Magnification is  $\times 25$



root biomass of *E. polystachya* after 70 days growing in soil contaminated with Urucu crude oil. This shows that this parameter is not a good endpoint for this species when exposed to petroleum products.

These results indicate that Urucu's crude oil cause more severe effects on *E. polystachya* than on *E. crassipes*. While after 91 days of exposure, the median lethal dose estimated for *E. crassipes* was 1.24 l m<sup>-2</sup>, in *E. polystachya* only 4 days of exposure were necessary to reach a similar mortality level in 0.47 l m<sup>-2</sup>. Besides differences in the sensitivity of both species, the substratum they occupy may be of importance. In the aquatic system, only the soluble part of the oil will reach the water column interacting with the roots, while a fluctuating layer that does not dissolve in the water column is formed (Val & Almeida-Val, 1999). On the other hand, the insoluble part of the oil penetrates in the soil, eventually reaching the root system where the pollutant becomes available for absorption by the plants. The implication of such differences in the case of an oil spill in varzea ecosystems is that crude oil could cause immediate damage to *E. polystachya*, while to *E. crassipes* damage would be evident only after a prolonged exposure.

Experiments performed with the oral ingestion of Urucu's crude oil by *Hoplosternum littorale* (tam-oatá), a facultative air breathing fish showed that only one ingestion of 3 ml kg<sup>-1</sup> of crude oil caused

physiological alterations; however, no death was observed during the exposure time (72 h) (Brauner et al., 1999). In the present study, during the same period of time, the death of 80% *E. polystachya* individuals submitted to dosages from 0.08 to 15.89 l m<sup>-2</sup> of crude oil occurred. Thus, even though crude oil may not be immediately lethal to fish, it would cause the loss of habitat and the accumulation of dead organic matter in the environment. The decomposition of this material will further decrease oxygen availability in varzea ecosystems. Heavy metals would also contaminate the dead organic matter (Osuji & Adesiya, 2005) and when decomposition takes place toxic elements may be incorporated into the trophic chains via the ingestion of plant material by invertebrates, preferential food items of several fish species.

Recently, concerns have arisen on the possibility of oil spills in the varzea, especially during the critical phase of recruitment for some key plant species of this ecosystem. Thus, what would be the implications of a high mortality of *E. crassipes* and *E. polystachya* in the case of a Urucu's oil spill accident? Emergent and floating plants are important to fish, wildlife, and humans. Several articles have summarized the value of these plants for fish and wildlife (Killgore et al., 1993; Mol et al., 2007; Pelicice et al., 2008). Together with fish like *Arapaima gigas* (pirarucu), an exclusive air breather, several Amazon fish feed on terrestrial invertebrates which inhabit aquatic macrophytes

during the period of high waters, such as the *Parauchenipterus galeatus* (cangati), the *Mylossoma duriventre* (pacú-manteiga), and *Triplophysa elongatus* (sardinha) (Claro-Jr. et al., 2004). The contamination of leaves of aquatic macrophytes with crude oil would cause the contamination of fish with crude oil. Other animals, such as the manatee (*Trichechus inunguis*), use *E. polystachya* (13.8%) and *E. crassipes* (11.2%) among their most important feeding items (Colares & Colares, 2002) and therefore, the death of these plants could cause a drastic reduction in the feeding options for this mammal. Due to their dense mass of roots, *E. crassipes* provide habitat for fish, fingerlings, insects, and other aquatic organisms (Junk & Howard-Williams, 1984). Humans also benefit from these plants because groups of emergent plants and free-floating plants dampen the force of waves which reduces shoreline erosion (Piedade & Junk, 2000). Furthermore, one of the most concerning effects of these plants' death would be the incorporation of a large quantity of dead biomass to the system, coupled with the amount of carbon that would no longer be fixed, estimated by Piedade et al. (1991) as 3.99 kg m<sup>-2</sup> of carbon. Thus, an oil spill in the varzea ecosystem causing damage to the flora would lead to direct and indirect damages to the fauna in these environments, especially the fish fauna, due to increased organic load and surface modification at the air/water interface, oxygen ,and habitat reduction.

## Conclusion

Concentration and exposure time are two important factors in the determination of the effect of crude oil on *E. polystachya* and *E. crassipes*. The determination of median LD<sub>50</sub> showed to be an effective method in evaluating the influence of these factors on the vegetation of ecosystems exposed to the risk of contamination by this pollutant.

When exposed to low dosage, *E. crassipes* survived for a long period of time, however, exposure to *E. polystachya*, for only a few days led to high mortality. Although *E. crassipes* survived a long period of time in a contaminated environment, plants were extremely affected by crude oil; this could be observed by the morpho-anatomical modifications in the leaves.

Higher concentrations of crude oil from Urucu caused a high mortality to *E. crassipes* and *E. polystachya*, whereas at low concentrations an inhibition of the growth of these species occurred. Further studies should be conducted to clarify the modifications in the metabolism of the plants responsible by growth inhibition. For instance, measures of photosynthetic rates and the quantification of nutrients and heavy metals in the living biomass and substratum could be studied.

The Amazon region depends on river transport for a large part of its trade, including oil transportation. On the other hand, the varzea ecosystems are very important areas for the regional economy owing to activities in fisheries, agro-forestry, and tourism. Oil spills in the Amazon/Solimões River could certainly have a negative impact on the varzea biota and ecosystem for they may compromise the population of key plant species of these floodplains. Therefore, long-term investigations on the effects and control of spills are crucial in helping the conservation, biodiversity, and sustainable management of these environments.

**Acknowledgments** This work is part of the GEOMA—Áreas Alagáveis Network Project, Brazilian Ministry of Science and Technology, MCT. Funding supporting this research was provided by INPA/Max-Planck Project, PPI 1090-5. Petrobras Oil Company provided oil through the PIATAM project. With thanks to Conselho Nacional de Pesquisas (CNPq) for Aline Lopes and Sonia Maciel Rosa-Osman masters Grants; CT-Petro Network (MCT/CNPq) for Aline Lopes Grant; INPA/Max-Planck Project, Laboratório de Ecofisiologia e Evolução (LEEM) and Laboratório Temático de Microscopia Óptica e Eletrônica for logistical and technical support.

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