Aquatic Ecosystem Health and Management 3 (2000) 471–477

Aquatic Ecosystem Health & Management

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Assessment of oil refinery waste on *Rhizophora mangle* L. seedling growth in mangroves of Todos os Santos Bay, Bahia, Brazil

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Abstract

Seeds of *Rhizophora mangle* L. were planted and comparatively assessed in six mangroves of Todos os Santos Bay, Bahia, Brazil. Five of the mangroves were on the north of the bay, an area under the influence of oil activities: two areas around the Landulpho Alves Refinery in Mataripe, and in Madre de Deus Island, Pati Island, and Fontes Island. The control site was a mangrove located on the south of the bay at Jiribatuba on Itaparica Island. In January and March, 1994, 1995 and 1998, sets of seeds were established and distributed in a pattern at regular intervals of 0.1, 0.5 and 1.0 m from one another. Every 3 months, the seedlings were assessed for fixation, height, number of lateral branches, surface area and wet and dry weight of leaves. During 1998, five individual seedlings were removed for biomass determination every 6 months. All variables were co-related with environmental conditions. After 12 months, a significant growth pattern difference ($p < 0.05$) was noted between seedlings from the mangrove around the refinery and those from Jiribatuba, mainly related to the rates of fixation higher than 50% and growth of leaves and precocious lateral branches to the two most impacted mangroves around the refinery, and Madre de Deus and Pati Islands. This branching was not observed on the seedlings grown in the mangroves of Fontes Island and Jiribatuba.

The construction of an effluent emission channel into one of the mangrove area from the refinery began in 1998. One hundred and twenty-five seedlings 1 yr in age and other young plants died following direct contact with these effluents in February, 1999. Published by Elsevier Science Ltd on behalf of AEHMS.

Keywords: Effluents; Refinery; Polycyclic aromatic hydrocarbons; Alcans

1. Introduction

Todos os Santos Bay is located at 12°39′40″S 38°34′30″W, in Bahia, Brazil. This bay is 927 km^2 in surface area, with an approximate volume of $6.39 \times 10^9\ m^3$, a medium depth of 6.9 m and currents with medium speed of $41.0\ cm\ s^{-1}$ ($1.476\ m\ h^{-1} = 0.8$ knots). These factors contribute to the low water residence time in the bay, minimizing the effects of the industrial effluents known to be discharged into the bay. Silva (1996) described the bay as a coastal body of water, strongly influenced by the masses of oceanic waters, varying in salinity from 28 to 36‰.

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1463-4988/00/$20.00 Published by Elsevier Science Ltd on behalf of AEHMS.
PII: S1463-4988(00)00042-7

In April 1992, an oil spill of 48,000 l occurred near the mangroves to the north of the bay, following the transference of crude oil ('Boscan', produced in Venezuela) between Petróleo Brasileiro Co. (PETROBRAS) containers in Madre de Deus Island. This oil has high viscosity (90,000 Speed Sedimentation Units) at 38°C and high concentrations of S and metals.

Machado (1996) established a system of points ranging from 0 to 55 corresponding to the level of hydrocarbon contamination. Points correspond to minimum and maximum contents of aliphatic hydrocarbons (*n*-alcans), polycyclic aromatic hydrocarbons (PAH), complex mixture not-resolved and crude petroleum as detected by gas chromatography in mangrove sediment samples.

In the mangrove around the refinery, Guedes (1996) recorded lower biomass compared to the other studied areas and she attributed this minimum to the dominance of young plants (2.5 m height). Here, the pH in the clay sediment is 5($\pm$0.5); there are high levels of Cationic Exchange Capacity (CEC), organic material (OM) and N, P, Cu, Zn. Machado (1996) detected heavy *n*-alcans, PAH, and lead and Cu in this sediment, shown to be most highly contaminated by hydrocarbons, i.e. assessed at 55 points. Silva (1996) recorded lower water transparency in this mangrove which was attributed to the high concentration of humic acids, high temperature values and concentrations of phenols, nitrate, ammonium and total solids in the surface water, which come from the refinery through the Mataripe river. Tavares (1996) also found 24.6 μg crude petroleum g^{-1} (a value corresponding to 41% petroleum) 1.14 μg PAH g^{-1} sediment and 15.8 μg *n*-alcans g^{-1} sediment in these sediments.

Recently, two refinery units were built to process physico-chemical and biological treatments of the refinery's effluent. A channel was built in 1998 for effluent emission into the mangrove area. Channel water is underlain by clay sediments and contains cyanobacteria and chlorophytes in abundance.

In Madre de Deus, a narrow band of vegetation occurs along the Madre de Deus Maritim Pier, which has been used to distribute petroleum since 1957. The sand and silt sediment is similar to the Pati and Jiribatuba mangroves (Guedes, 1996; Machado, 1996). Machado (1996) detected PAHs in the sediment, at the level of 30 points. In Pati Island, next to Madre de Deus, the oil is visible on the sediment and seedlings. Here, Machado (1996) detected grades of light alcans at 15 points. In Fontes Island, the vegetation band is also narrow and the sediment is composed of silt and thin sand. Machado (1996) found light alcans corresponding to 20 points. According to Machado (1996) and Silva (1996), the thin sand sediment result in particulate material increase. In Jiribatuba, Machado (1996) detected low levels of hydrocarbons at 5 points and attributed this to small boat motors in use in the channel of Itaparica Island and to hydrocarbons carried through the water from the north. In this area, the sand and silt sediment has low cation exchange capacity and little OM.

In this study, seedling development was analyzed to clarify which biometric parameters could be used as indicators of the environmental quality of mangrove areas influenced by chronic oil spillage. Morphophysiological variations of the initial growth of *Rhizophora mangle*L. seedlings were evaluated in relation to oil activities in the Todos os Santos Bay. Five mangroves are located in the north of Todos os Santos Bay, where PETROBRAS petroleum activities are concentrated. Two of these areas are close to the Landulpho Alves Refinery of Mataripe which has refined and distributed petroleum products since 1950. Another three were located at Madre de Deus Island, Pati Island and Fontes Island. The mangrove of Jiribatuba (Itaparica Island) in the south was used as an 'in-bay' control site It is exposed to similar environmental conditions but is free of the direct influence of petroleum activities.

2. Methodology

Seeds of *R. mangle* were collected and planted in each area, adjacent to mangrove vegetation, 5 cm deep in the sediment and in patterns at regular intervals of 0.1, 0.5 and 1.0 m from one another.

In February 1994, sets were established with 90 seeds adjacent to the refinery; 100 seeds on Madre de Deus Island; 150 seeds respectively on Pati and Fontes Islands; and 125 seeds in Jiribatuba. In March 1995, other sets were established: 60 seeds close to the refinery, 165 seeds in Pati Island, 20 seeds in Fontes Island and 180 seeds in Jiribatuba.

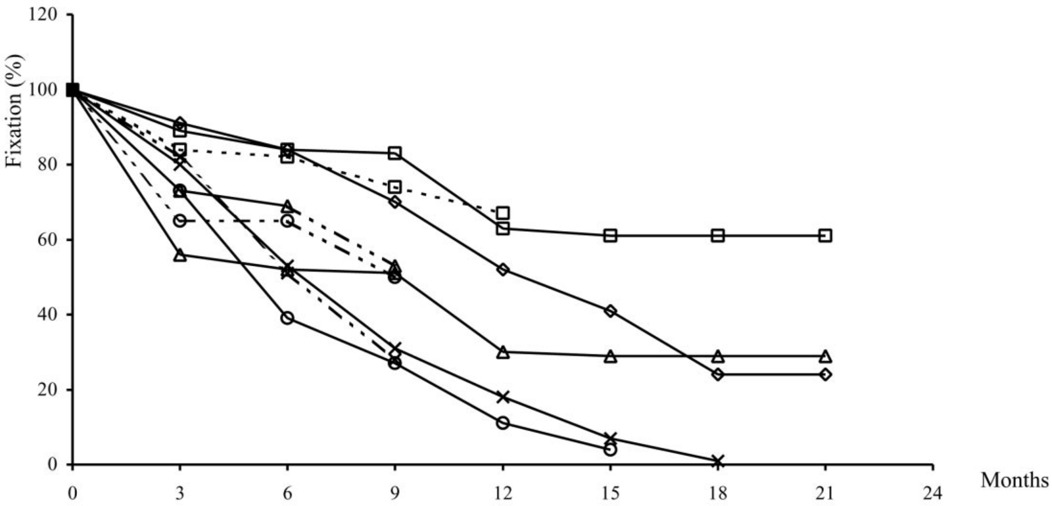


Fig. 1. Fixation (%) for seedlings of *R. mangle* in mangroves of Todos os Santos Bay, Bahia, Brazil: 1994 (—), 1995 (· · – · ·) and 1998 (···), –□– Refinery of Mataripe, –◇– Madre de Deus Island, –△;– Pati Island, –○– Fontes Island and – × – Jiribatuba (Itaparica Island).

All these seedlings were assessed until December 1995. In January and March 1998 two additional sets of 100 seeds were established in a new and deforested mangrove area around the refinery. These seedlings were assessed until March 1999.

Every 3 months, all seedlings were assessed for establishment in the sediment fixation (%), height (cm) and number of lateral branches and leaves. The height of each individual was measured from the stem base to the nodes of the last leaves. Samples of mature leaves from the middle node were collected and taken to the laboratory, where they were washed, dried, weighed for wet mass. Leaves were outline-drawn on white paper to determine surface area. Subsequently samples were dried at 60–100°C for 5 days, and dry mass assessed. Every 6 months, from the seedlings planted in 1998 on the new mangrove area adjacent to the refinery, five individuals were taken for biomass determination.

Data were analyzed by the Friedman variance no-parametric test ($p < 0.05$) and plotted in a rank from 0.00 to 5.00 where the most important morphological parameters (e.g. height, lateral branches and size of leaves) could be more correlated to the environmental condition (e.g. gradients of oil pollution). A maximum of 5.00 rather than 10.00) was used because other unstudied variables in the same area could affect the seedlings' growth.

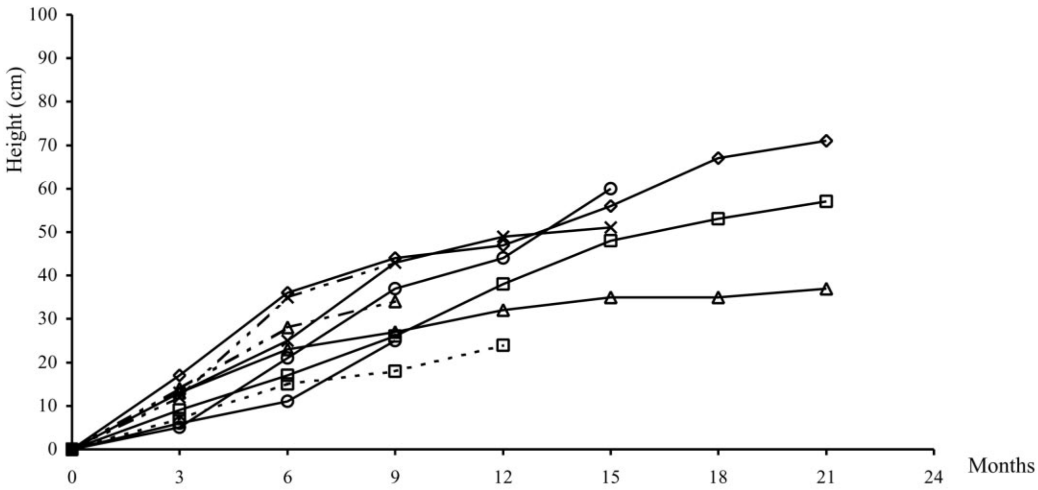
3. Results and discussion

R. mangle seedling growth was studied in relation to the potential influence of oil pollution. In comparison to the adult plants studied by Guedes (1996) in the same seasonal period, no statistical difference was found between the mangroves around the refinery and those on the Jiribatuba control site. The best seedling parameters were fixation, height, number of lateral branches and size of leaves.

Getter et al. (1985), who studied *R. mangle* seedlings from polluted mangroves, considered their high rate of growth, high leaf number and resistance as evidence of chronic stress. They showed that *R. mangle* was more resistant to the oil than other species.

3.1. Fixation of seedlings

The fixation of seedlings of *R. mangle* was a health indicator for the most impacted mangroves around the refinery in two different cases. In one area, under chronic oil pollution, rates of fixation were higher than 60%, at 1 and 2 yr of age (Fig. 1). In another area, 125 one year old seedlings and other young plants which had been successfully established, died following direct contact in 1999 with the effluents from the newly constructed emission channel. In the



Mangroves	Refinery Mataripe	Pati Island	Fontes Island	Jiribatuba	Madre de Deus Island
Global rank	1.60	2.00	2.80	4.00	4.60

Fig. 2. Height (cm) for seedlings of *R. mangle* in mangroves of Todos os Santos Bay, Bahia, Brazil. Time and symbol designations as in Fig. 1. For global ranking, $F = 7.6279$ and $P = 0.0012$. Values linked by the same line do not differ significantly.

first case, seedlings grow more slowly in height but faster in root wet biomass (50 g yr^{-1}) which contributed to the high rate of fixation. The clay sediment contributed to high fixation on the mangrove close to the refinery of Mataripe. On the other hand, this environmental variable increased the time of pollutant residence near the seedlings. A high rate of fixation of *R. mangle* seedlings at (80% after 1 yr) has been observed in studies of impacted Brazilian mangroves in Baixada Santista/Cubatão (Menezes et al., 1994), and in Bertioga Channel (Rodrigues et al., 1995) both in São Paulo.

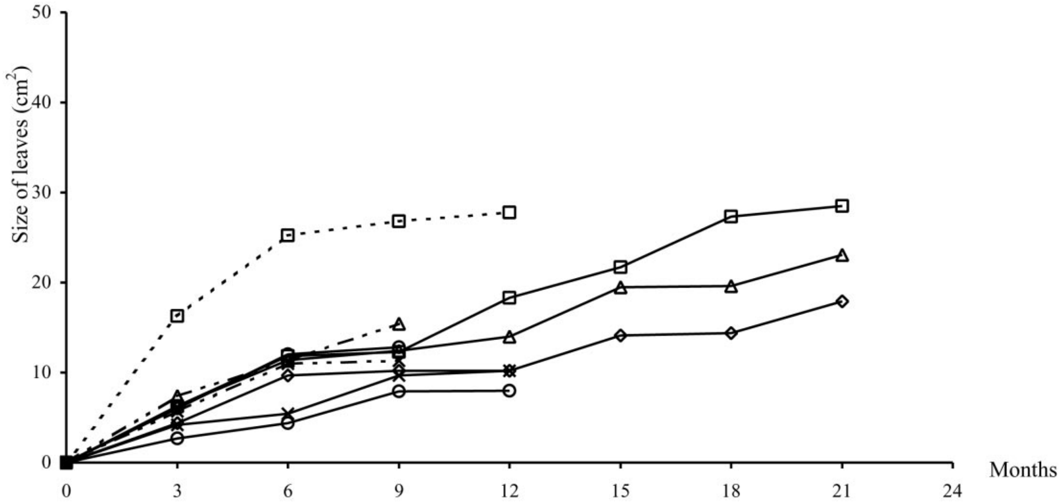
In Madre de Deus Island, a fixation rate of less than 50% was observed after 2 yr and in Pati Island of 50% after 1 yr. It is likely that the sand sediment allowed good seedling fixation and its minerals did not interact so markedly with the hydrocarbons which were in low concentration (Machado, 1996). Possibly, they were flushed out by the water due to the mangrove location in the bay.

At Fontes and Jiribatuba Islands, the rates of fixation were the lowest. At Fontes, the high amount of particulate material against the stems of seedlings seemed to have a negative effect on their fixation which was less than 30% after 1 yr and 0% after 2 yr (Fig. 1). Odum and Johannes (1975) noted that seedlings can die when covered by tidal sediment and that superficial roots are damaged when gas exchange is interrupted. At Jiribatuba, the control site, seedlings were crushed by local people and the fixation rate was less than 35% after 1 yr, decreasing to 0% in 2 yr (Fig. 1), due mostly to intense oyster collection activities.

The placement of seeds at intervals of 0.1–1.0 m among the seedlings did not interfere with their fixation which was determined rather by sediment type (Fig. 1).

3.2. Growth of seedlings in height, lateral branches and size of leaves

The mangrove seedlings most impacted by hydrocarbons from the refinery of Mataripe showed a slow primary growth in height (Fig. 2). However, the same seedlings presented a more precocious secondary



Mangroves	Fontes Island	Jiribatuba	Madre de Deus Isl.	Pati Island	Refinery Mataripe
Global rank	1.00	2.25	2.75	4.25	4.75
Average	5.75	7.38	11.57	15.17	18.03

Fig. 3. Size of leaves (cm^2) for seedlings of *R. mangle* in mangroves of Todos os Santos Bay, Bahia, Brazil. Time and symbol designations as in Fig. 1. For global ranking, $F = 37.0000$ and $P = 0.0000$. Values linked by the same line do not differ significantly.

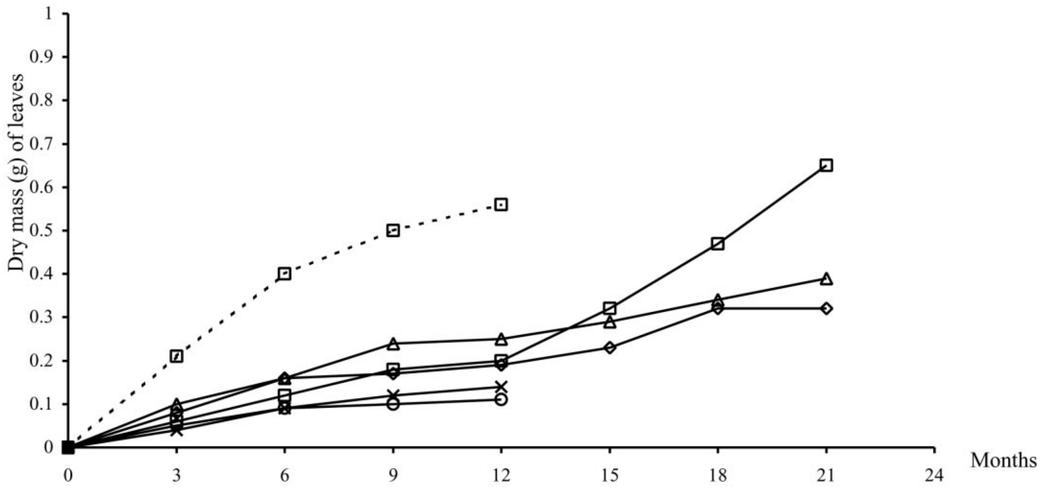
growth in diameter of lignified stem and lateral branches, indicating they matured more quickly.

In the two mangrove areas adjacent to the refinery and most impacted by oil activities (15 μg *n*-alcans g^{-1} sediment and 23 μg crude oil g^{-1} sediment, Machado, 1996), the seedlings showed the smallest growth in height (Fig. 2) in relation to the highest rates of fixation; but an average of five lateral branches and larger size and mass of leaves (Figs. 3 and 4) were co-related to the high levels of hydrocarbons in the sediment.

The precocious lateral branches of seedlings began to appear at 6 months of age in the most impacted mangroves which received effluents from the refinery. These mangroves differed statistically ($p < 0.05$) compared to the other five. The growth of lateral branches began to differ significantly after 1 yr to this mangrove in comparison to Madre de Deus and Pati Islands, where the seedlings began to form two lateral branches after only 1 yr. Such lateral branches were not found on the seedlings from Fontes Island and Jiribatuba even after 2 yr. In a non-impacted Brazilian mangrove in Maragogipe, state of Bahia, there was no precocious lateral branches in seedlings of the same age.

When the growth of lateral branches increased, the number of leaves began to differ significantly after 1 yr on the mangrove around the refinery in comparison to Madre de Deus and Pati Islands. The seedlings distributed at 0.5–1.0 m produced bigger leaves than those distributed at 0.1 m, probably due to competition for space into the set (Figs. 3 and 4). The variation in weight and size of leaves resulted in 'heavier' leaves and more precociously mature seedlings in the most impacted mangroves around the refinery. Rodrigues et al.(1989) recorded the variable surface area of leaves as a parameter indicating oil pollution in Brazilian mangroves from Baixada Santista, state of São Paulo.

Although Machado (1996) had shown presence of light alcans on Fontes Island, the presence of these hydrocarbons was not analyzed in seedling tissues. However, this study indicated a relation between the contaminants and the death of seedlings younger than 2 yr, because the statistical evidence of the data was similar to that for plants killed by the refinery



Mangroves	Fontes Island	Jiribatuba	Refinery Mataripe	Madre de Deus Isl.	Pati Island
Global rank	1.33	1.67	3.33	4.00	4.67
Average	0.10	0.13	0.32	0.23	0.28

Fig. 4. Dry mass (g) of leaves for seedlings of *R. mangle* in mangroves of Todos os Santos Bay, Bahia, Brazil. Time and symbol designations as in Fig. 1. For global ranking, $F = 10.8571$ and $P = 0.0026$. Values linked by the same line do not differ significantly.

effluents. According to Baker (1971a), light alcans can penetrate seedling tissue quickly, causing metabolic damage. Vargas (1983) considered that if the contact of plants with oil is gradual and in low concentration, they survive and become adapted to the new environmental condition. Lewis (1983) even considered that they use oil as an energy source. Getter et al. (1983) studied the effects of different combinations of oils on *R. mangle* seedling leaves and showed that lateral branch growth increased in the presence of less than 500 $\mu l\ l^{-1}$ of oil (with its dispersing 'Fuel N2D'). They studied mangrove seedlings which appeared to perform well with application of the crude oil 'Light Arabian', indicating possible adaptation to spilled oil and suggesting some specificity between mangrove species and oil type. Getter et al. (1985) considered the growth of lateral branches as an effect of sublethal stress in *Avicennia germinans* exposed in the laboratory to oil, and concluded that low oil concentrations have a stimulating effect on seedling growth. Baker (1971a) treated some plants with oil and recorded the increase of growth in *Festuca rubra* and of dry mass in *Puccinellia* sp. after oil degradation. As well, he treated the green algae *Bostrychia* sp. with oil and they died. Baker (1971b), after evaluating the seasonal effects of crude oil (Kuwait) on some estuarine plant species, noted that this oil type caused more damage to plants in warmer climates, because higher temperatures reduce oil viscosity and allow for faster tissue penetration. The effects seem to be specific for each oil type.

Chronic pollution has not inhibited the growth of the seedlings in the mangroves around the refinery to date, and they seem to be adapted. However some emissions of effluents may cause serious and high rates of damage as recorded in this study of mangroves that received effluents from the refinery in the summer.

4. Conclusions

R. mangle seedlings have been shown to be appropriate and sensitive bio-indicators to the poor condition of mangrove stands subjected to chronic oil pollution around a refinery. The best indicators of effect were fixation, growth as height, number of lateral branches and size of leaves.

Precocious lateral branches of seedlings began to appear at 6 months of age in the most impacted mangroves which received effluents from the refinery.

The variation in weight and size of leaves resulted in 'heavier' leaves and precocious ageing in seedlings of the most impacted mangroves around the refinery.

The chronic pollution had not inhibited the growth of the seedlings in the mangroves around the refinery to date, and they seem to be adapted to the presence of oil constituents. However, the emissions of effluents may cause serious and high rates of damage.

Acknowledgements

We thank Denis L. Filer (University of Oxford/ Forestry Institute) for the English review of the manuscript.

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