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ORIGINAL ARTICLE

Effects of automotive diesel oil on germination of *Avicennia germinans* and *Laguncularia racemosa* mangrove propagules

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ABSTRACT

Mangrove ecosystems are sensitive to oil, as spills can impair developmental processes of mangrove vegetation. Since the 2010s, the Brazilian equatorial margin, more specifically the Pará-Maranhão Basin and the mouth of the Amazonas River, has been affected by oil runoff from urban activities and the increased risk from exploratory deepwater drilling for oil extraction. Dispersal of mangrove propagules occurs during the tidal cycles, when the presence of tensors in the water can affect germination. We analyzed the effects of diesel oil on the germination of propagules of the two most common mangrove species in the region, *Laguncularia racemosa* and *Avicennia germinans*, in six treatments of diesel oil in the water (0.5%, 1%, 1.5%, 2%, 3% and 4%) and a diesel-free control. The response variables were germinability (G%), mean germination time, mean germination speed and the germination speed index (GSI). G% and GSI in *L. racemosa* propagules differed significantly between the control and all treatments (G% and GSI < 10% in the 3% treatment). Propagules of *A. germinans* were more resistant, and the physiological variables did not differ significantly among treatments and control (G% > 90% in all treatments). Our results indicate that, at the germination stage, *L. racemosa* was more susceptible than *A. germinans* to contamination by automotive diesel oil.

KEYWORDS: hydrocarbons, environmental tensors, Brazilian Equatorial margin, ecophysiological responses

Efeitos de óleo diesel automotivo sobre a germinação dos propágulos de mangue de *Avicennia germinans* e *Laguncularia racemosa*

RESUMO

Os ecossistemas de manguezais são sensíveis ao óleo, pois os derramamentos podem prejudicar os processos de desenvolvimento da vegetação de mangue. Desde a década de 2010, a margem equatorial brasileira, mais especificamente a Bacia do Pará-Maranhão e a foz do Rio Amazonas, vem sendo afetada pelo vazamento de petróleo proveniente de atividades urbanas e pelo aumento do risco de perfurações exploratórias em águas profundas para extração de petróleo. A dispersão dos propágulos do manguezal ocorre durante os ciclos das marés, quando a presença de tensores na água pode afetar a germinação. Analisamos os efeitos do óleo diesel na germinação de propágulos de duas espécies de mangue mais comuns na região, *Laguncularia racemosa* e *Avicennia germinans*, em seis tratamentos de óleo diesel na água (0,5%, 1%, 1,5%, 2%, 3% e 4%) e um controle sem óleo diesel. As variáveis resposta foram germinabilidade (G%), tempo médio de germinação, velocidade média de germinação e índice de velocidade de germinação (IVG). G% e IVG dos propágulos de *L. racemosa* diferiram significativamente entre o controle e todos os tratamentos (G% e GSI < 10% no tratamento 3%). Os propágulos de *A. germinans* foram mais resistentes e as variáveis fisiológicas não diferiram significativamente entre tratamentos e controle (G% > 90% em todos os tratamentos). Nossos resultados indicam que, no estágio de germinação, *L. racemosa* foi mais suscetivel que *A. germinans* à contaminação por óleo diesel automotivo.

PALAVRAS-CHAVE: hidrocarbonetos, tensor ambiental, margem Equatorial Brasileira, respostas ecofisiológicas

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INTRODUCTION

Mangroves are among the most productive ecosystems in the world, providing important ecosystem services and wide connectivity with other coastal ecosystems such as coral reefs, marine prairies, as well as a continental-ocean interface (Yokoya 1995; Mochel 2011; ICMbio 2018; Kristian and Oktorie 2018; Van Der Stocken 2019). In 2018, global mangrove coverage was estimated at 150,000 km^2 and nearly 25% was damaged by human actions, mainly deforestation, pollution, coastal zone occupation, shrimp cultivation and climate change (Ferreira and Lacerda 2016; Kristian and Oktorie 2018). Brazil has approximately 10,020 km^2 of mangrove forest, 77.6% of which are in the Amazon coastal zone of the Brazilian states of Maranhão, Pará and Amapá (Diniz *et al*. 2019).

Oil pollution is one of the most recurrent impacts on coastal and marine systems due to accidents with oil tankers, offshore exploratory activities, leaks of fuel or lubricant from vessels, all of which damages the adjacent ecosystems (Thompson and Thompson 2020) and human health (Pena *et al*. 2020). Oil pollution can also occur during port operations with anchored vessels, from leaks in pipelines (Matos *et al.* 2019), during vessel cleaning (Alcântara and Santos 2005), leaks from stored fuels in tanks at gas stations (Finotti *et al.* 2001), pipeline, road and rail transport (Martins and Mochel 2022).

The impact of oil on coastal environments depends on the amount of spillage, the type of oil and its toxicity, and environmental conditions such as the predominant type of sediments, tidal circulation and prevailing climatic conditions at the time of contact with the oil (Soares *et al.* 2006; Chequer *et al.* 2017). Acute and chronic effects result from impacts of different types of oil in mangrove ecosystems (Hoff and Michel 2014). Diesel oil is the most consumed fuel in Brazil (Petrobras 2021).

Mangroves on the Amazonian coast are largely pristine, although areas near large urban centers such as the capital cities of Belém (Pará state), Macapá (Amapá), and São Luis (Maranhão), all located within the delta complex of the Amazonas River, may be heavily affected by urban pollution, including oil runoff. There are records of a diesel oil spill in São Marcos Bay (Maranhão) after the collision of a ship with a shipwreck stranded on a sandbar in 1990, and of diesel oil leakage in a mangrove during the fueling process of mining-train locomotives in 2000, among other occurrences (Martins and Mochel 2022). Since the 2010s, the Brazilian equatorial margin has increasingly been the target of development plans and exploratory deepwater drilling for oil and natural gas extraction, raising the risk for possible oil leaks from activities related to these operations in the fragile and lush areas of mangroves on the Amazon coast (Silva Junior and Magrini 2014).

Mangrove tree species have viviparous reproduction as an inherent characteristic and propagule dispersal occurs through the tides (Hutchings and Saenger 1987; Elmqvist and Cox 1996; Tomlinson 2016). Dispersion success depends on the buoyancy and durability of propagules, as well as on their hydrodynamics and mortality from predation and damage caused by crabs and insects (Kathiresan and Bingham 2001; Hoff and Michel 2014; Hogarth 2015). It is in this highly vulnerable phase of the plant that the presence of tensors in the water can affect germination. Oil contamination can harm the germination process, as well as the growth, development and establishment of mangrove seedlings (Duke *et al.* 1998; Chindah *et al*. 2011; Hoff and Michel 2014; Chequer *et al.* 2017).

Previous studies with mangrove species in contact with various types of petroleum derivatives and crude oil were developed with the propagules planted directly in the sediment or transferred at the mangrove seedling stage and subsequently exposed to the contaminant (Zhang *et al.* 2007; Naidoo *et al.* 2010; Nardes *et al.* 2013; Amin *et al.* 2017; Chequer *et al.* 2017; Guedes *et al.* 2018). In the present study, we evaluated the germination of propagules submitted to different percentages of contamination of estuarine water with diesel oil under laboratory conditions, aiming to analyze the effects of the contaminant on the first stage after propagules have left the trees. Among the mangrove species that occur on the Amazonian coast, we opted to work with the two most common and widely distributed ones, the black mangrove, *Avicennia germinans* (L.) L., locally known as *siriba* or *siriúba*, and the white mangrove or inkworm, *Laguncularia racemosa* C.F. Gaertn.

MATERIAL AND METHODS

Study area and material acquisition

The study was carried out on Maranhão Island (Maranhão State, Brazil), where mangroves are composed of *Avicennia germinans*, *Avicennia schaueriana* Stap. & Leech. ex Mold., *Laguncularia racemosa*, *Rhizophora mangle* L. and *Conocarpus erectus* L. (Rebelo-Mochel 1997; Mochel 2011). Mangrove propagules from *A. germinans* and *L. racemosa*, as well as the estuarine water used for the experiment, were collected at the Mangue Seco mangrove, in the municipality of Raposa (2º27'06.86"S, 44º09'20.33"W to 2º27'21.81"S, 44º09'45.76"W) (Figure 1).

The collection was made during high tide. This site is quite pristine and regularly used as a research site by the authors, being far from the municipal headquarters, where a community of fishermen lives from shrimp and shellfish fishing. The propagules were identified according to the specifications of each plant of the species: *A. germinans* – light propagule, its length varies from 25 mm to 30 mm, *L. racemosa* – light propagule, its length varies from about

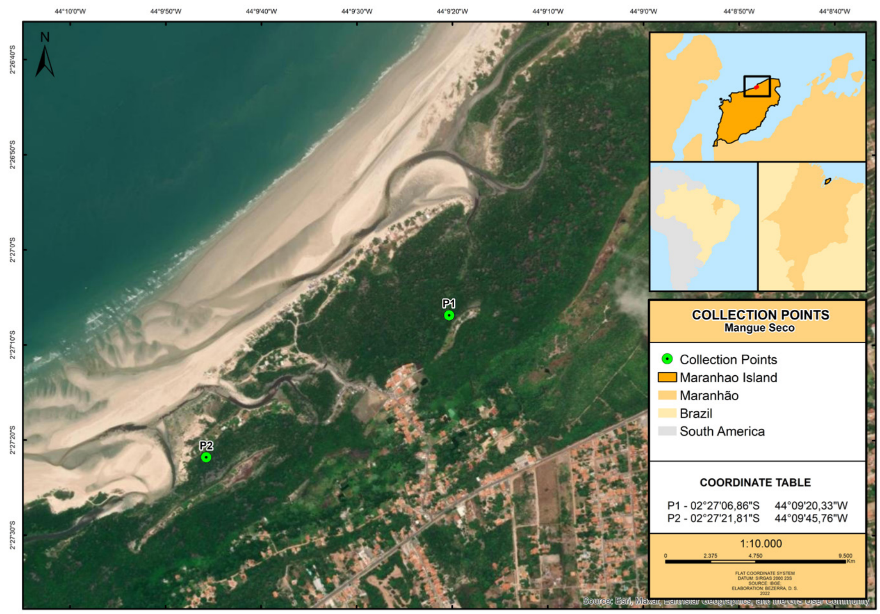

Figure 1. Location of the collection points of *Laguncularia racemosa* and *Avicennia germinans* propagules in the Mangue Seco mangrove, Ilha do Maranhão, Maranhão, Brazil. This figure is in color in the electronic version.

11.0 mm to 19.5 mm. Propagule collection and selection criteria followed the recommendations of Goforth and Thomas (1980) and Mochel and Fonseca (2019), to select healthy propagules with greater possibility of developmental success. The experiment was carried out at the Mangrove Recovery Center (CERMANGUE) of the Department of Oceanography and Limnology at the Federal University of Maranhão (Universidade Federal do Maranhão, UFMA).

Germination

To observe the effect of the automotive diesel oil on propagule germination in both species, we simulated an acute contamination event with six treatments: 0.5% (T_1); 1% (T_2); 1.5% (T_3); 2% (T_4); 3% (T_5) and 4% (T_6) and a control (C) without oil, for each species, based on the study by Kim (2014) who used 0%, 6%, 12% and 18% and Chequer *et al.* (2017) who used 3%. The experiment was carried out in triplicate, each replicate on a different date. Each replicate consisted of one glass bowl containing 500 mL of estuarine water at 28 g kg^{-1} of salinity for each species and oil percentage. Salinity was measured with a portable refractometer (Quimis Q767-3, Shanghai Precision & Scientific Instrument Co., Shanghai, China). For each bowl, we used 36 propagules of *A. germinans* and 114 propagules of *L. racemosa* (Figure 2).

The final amount of propagules used in the experiments varied (1) with the availability of parent trees in the study area, and (2) after sorting the healthy propagules in the laboratory. The propagules of *A. germinans* germinated from the 1st to the 6th day and those of *L. racemosa* germinated from the 1st to the 13th day of the experiment. Propagules were randomly distributed among replicates and were not grouped by size or weight. Propagules were considered germinated when they presented the hairy hypocotyl emission in *A. germinans*, and root protrusion with at least 1 cm in *L. racemosa*.

The following variables were evaluated: (1) germinability (G%), calculated as %G = (Σni /N-1).100, where Σni is the total number of germinated propagules per day in relation to the number of propagules put to germinate (N); (2) mean germination time (MGT, measured in days), calculated as T = Σ ni.ti / Σ ni, where ni = number of propagules germinated per day, and ti = incubation time; (3) mean germination speed (MGS. $days^{-1}$), calculated as V = 1/t = Σ ni / Σ ni.ti, where t = mean germination time, ni = number of propagules, ti = incubation time; and (4) germination speed index (GSI), calculated as GSI = (G1/T1) + (G2/T2) + (G3/T3)... + (Gi/

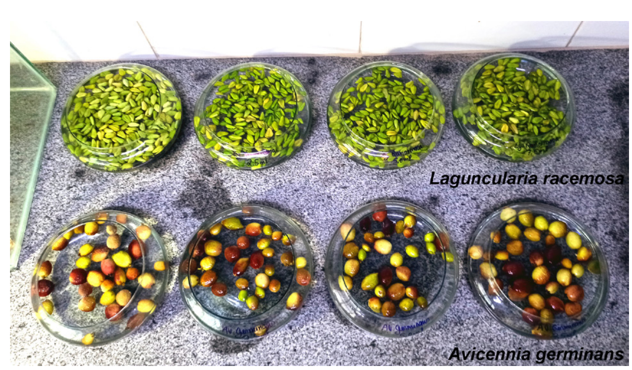

Figure 2. Distribution of *Laguncularia racemosa* and *Avicennia germinans* propagules in the experimental vials containing estuarine water (500 mL) and different percentages of diesel oil. This figure is in color in the electronic version.

Ti), where G1 to Gi = number of germinated propagules each day, and T_1 to Ti = time in days of the experiment (each day) (Maguire 1962; Borghetti and Ferreira 2004).

Statistical analysis

The Levene test was used to evaluate the homogeneity of variance. Variables that met the assumption for parametric analysis were compared among treatments and the control with ANOVA. Significant results were compared by the post-hoc Tukey test for pairwise comparison of the means. Variables with non-parametric distribution were analyzed with a Kruskal-Wallis test followed by Mann-Whitney tests for pairwise comparison. To further evaluate the association of the germination variables with the oil treatments in each species, we used principal component analysis (PCA) based on a correlation matrix (Valentin 2012; Legendre and Legendre 2012). The statistical significance of the difference between the groups obtained by the ordination technique was assessed with a one-way PERMANOVA test (Anderson 2001). The statistical analyses were performed with the Past 4.03 software (Hammer *et al*. 2001) with a significance level of 0.05 for all tests.

RESULTS

Laguncularia racemosa showed a significant difference among treatments for G% (F = 4.15; df = 6/14, p = 0.013), with significantly higher G% in the control and no difference among replicates within treatments (rejoinder) (Table 1).

Table 1. Physiological variables of germination of *Laguncularia racemosa* and *Avicennia germinans* propagules (mean ± standard deviation of three replicates) subjected to different percentagens of diesel oil 0.5% (T1); 1% (T2); 1,5% (T3); 2% (T4); 3% (T5); 4% (T6) and 0% (C-control). G% = germinability; MGT = mean germination time; MGS = mean germination speed; GSI = germination speed index. Different letters in the column within species indicate significant pairwise differences between treatments and control (C) according to a Tukey test.

Diesel oil treatment	G%	MGT (days)	MGS (days)	GSI
		Laguncularia racemosa		
C	80.8 ± 30.8 ^{a}	5.2 ± 0.5 ^{b}	0.2 ± 0.0 ^{b}	94.1 ± 27.1 ^{a}
T_1	24.1 ± 16.7 ^{b}	6.6 ± 3.7 ^{b}	0.1 ± 0.1 ^{b}	22.8 ± 8.8 ^{b}
T_2	22.2 ± 20.3 ^{b}	6.2 ± 3.3 ^{b}	0.2 ± 0.2 ^{b}	26.8 ± 12.2 ^{b}
T_3	24.5 ± 19.3 ^{b}	6.4 ± 0.4 ^{b}	0.2 ± 0.0 ^{b}	22.1 ± 10.7 ^{b}
T_4	13.2 ± 3.8 ^{b}	6.1 ± 3.7 ^{b}	0.2 ± 0.1 ^{b}	9.4 ± 4.7 ^{b}
T_5	8.8 ± 9.0 ^{b}	8.1 ± 5.5 ^{b}	0.1 ± 0.1 ^{b}	5.5 ± 2.5 ^{b}
T_6	15.4 ± 6.3 ^{b}	6.5 ± 3.0 ^{b}	0.1 ± 0.1 ^{b}	9.2 ± 5.0 ^{b}
		Avicennia germinans		
C	98.1 ± 2.9 ^{b}	1.6 ± 0.3 ^{b}	0.6 ± 0.1 ^{b}	74.9 ± 7.2 ^{b}
T_1	96.2 ± 7.0 ^{b}	1.6 ± 0.3 ^{b}	0.6 ± 0.1 ^{b}	75.2 ± 8.6 ^{b}
T_2	95.1 ± 4.7 ^{b}	2.0 ± 0.6 ^{b}	0.5 ± 0.2 ^{b}	61.8 ± 9.1 ^{b}
T_3	98.1 ± 1.6 ^{b}	2.1 ± 0.5 ^{b}	0.5 ± 0.1 ^{b}	61.9 ± 6.2 ^{b}
T_4	98.2 ± 2.9 ^{b}	2.3 ± 0.6 ^{b}	0.4 ± 0.1 ^{b}	69.1 ± 7.9 ^{b}
T_5	99.1 ± 1.4 ^{b}	1.7 ± 0.4 ^{b}	0.6 ± 0.1 ^{b}	78.6 ± 9.1 ^{b}
T_6	96.4 ± 3.8 ^{b}	2.0 ± 0.3 ^{b}	0.5 ± 0.1 ^{b}	75.8 ± 7.1 ^{b}

Avicennia germinans, showed no significant difference in G% among the treatments and the control (F = 0.42; df = 6,14, p = 0.85) (Table 1). GSI varied significantly in *L. racemosa* (F = 6.20; df = 6,13, p = 0.002), with all treatments presenting significantly lower values than the control (Table 1). No significant difference was observed for GSI in *A. germinans* (F = 0.25; df = 6/14, p = 0.95). No significant variation was observed among treatments and control for MGT and MGS in both species (Table 1).

Axis 1 and 2 of the PCA explained 46% and 35% of the data variance, respectively. G%, GSI and MGS of *L. racemosa* were positively correlated with axis 1, being associated with the control. MGT was negatively correlated with axis 1, being associated with treatments with higher concentrations of diesel oil (T_4, T_5 and T_6). MGS and GSI of *A. germinans* were positively correlated with axis 2, being associated with the control and T_1, while MGT was negatively correlated with axis 2, with association with treatments T_2, T_3 and T_4, and G% was close to the origin of the graph, indicating low representativeness in the sample, and little association with the treatments (Figure 3). The PERMANOVA indicated significant differences (F = 99.09; p = 0.01) among the control (CG) and the treatments with low T_1, T_2 and T_3) and high (T_4, T_5 and T_6) oil concentrations.

DISCUSSION

Our results showed that *A. germinans* and *L. racemosa* responded differently when exposed to diesel oil. *Avicennia germinans* exposed to crude oil in concentrations of 15 ml and 120 ml showed retardation in seedling development (Chindah *et al.* 2011), and the development and survival of planted propagules of *A. schaueriana*, *R. mangle* and *L. racemosa* exposed to 3% of marine diesel oil was significantly affected (Chequer *et al.* 2017).

Under unfavorable conditions, the seed germination process can be hampered (Baskin and Baskin 2014), which seemed to have been the case for *L. racemosa*, indicating that the species is sensitive to contact with diesel oil in the germinative stage, reinforcing the evidence from other studies that the components of petroleum derivatives or crude oil can inhibit both the germination process and the development of several mangrove species (Zhang *et al.* 2007; Hoff and Michel 2014; Guedes *et al.* 2018).

There was a significant variation in the germination speed index in *L.racemosa*, with greatest germination capacity of the propagules in the control, and decreasing capacity with the increase of diesel oil in the treatments. This same effect of decreasing germination capacity was also observed for halophytes from coastal wetlands exposed to diesel oil (Kim 2014).

Avicennia germinans showed no negative effects of diesel oil on any of the germination variables analyzed, but the

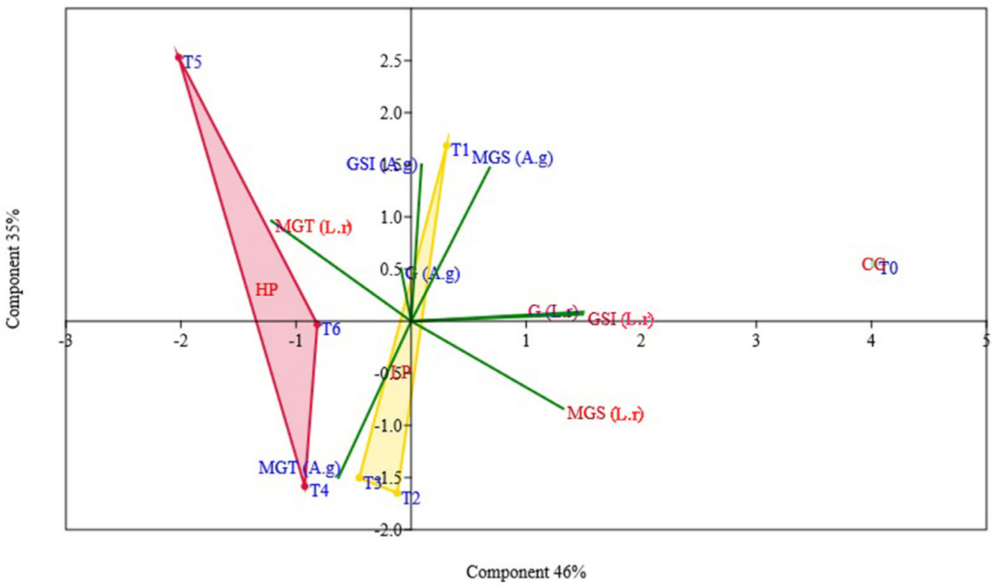

Figure 3. Principal component analysis (PCA) showing the correlation among germination response variables (G% = germinability; MGT = mean germination time; MGS = mean germination speed; GSI = germination speed index) for *Laguncularia racemosa* and *Avicennia germinans* with low percentages of diesel oil [LP: 0.5% (T1); 1% (T2); 1,5% (T3)], high percentages of diesel oil [HP: 2% (T4); 3% (T5), 4% (T6)] and the control group with no diesel oil (CG). This figure is in color in the electronic version.

absence of a significant effect does not allow us to speculate to what extent the species would be resistant to diesel oil percentages greater than 4%. The mangrove response to contact with oil depends on the amount and type of oil with which it is impacted (Chequer *et al.* 2017; Soares *et al.* 2006). It is also necessary to consider the developental stage of the mangrove when it is hit by the contaminant (stage of germination, seedlings or established tree). Studies conducted on *A. germinans* seedlings using different concentrations of lubricating oil and crude oil (15ml, 120ml) caused their death (Proffitt *et al.* 1995; Chindah *et al.* 2011), while in *Avicennia marina* (Forssk.) Vierh. and *Bruguiera gymnorrhiza* (L.) Lam., marine fuel oil (bunker) impaired the healthy development and caused seedling abnormalities (Naidoo *et al.* 2010). Although there is extensive literature about the negative effects of oil spills on mangrove ecosystems, reporting short and long-term effects, the action of oil on propagules in the germination stage, when they are dispersed in the water, is still little known.

CONCLUSIONS

The germination of *Laguncularia racemosa* was significantly lower than that of *Avicennia germinans* when exposed to diesel oil at percentagens between 0.5 and 4%. This indicates that this species is especially susceptible to diesel oil in the germination stage of the propagules, suggesting a greater degradation potential than other mangrove species in areas affected by diesel oil spills. *Avicennia germinans* proved to be resistant to the percentages applied in this study, and it is recommended to test this species with higher percentages of oil. Our results contribute to the knowledge on the sensitivity of propagules during the germination process to tensors and the general process of ecological recovery of mangroves degraded by diesel oil residues.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, Martins, J.C.S., upon reasonable request.