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Biodegradation of bioemulsified heavy oil in mangrove soil

Biodegradación de petróleo pesado bioemulsionado en un suelo de manglar

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ABSTRACT

When oil is released into coastal areas and estuaries, it adheres to the roots of trees and seedlings, causing their asphyxiation and death within a few days. To mitigate this problem, the restoration of mangrove soil contaminated with emulsified oil was evaluated through the application of bacterial surfactant produced by a strain of *Azospirillum lipoferum* and another surfactant produced from pine oil. *Risophora mangle* propagules were collected from a mangrove forest located in the community of El Bellote, Paraíso, Tabasco, Mexico and planted in artificial solonchak sandy soil. When the *R. mangle* plants were three months old, their soil was contaminated with emulsified oil. The heavy oil fraction content and number of colony-forming units were evaluated through bioassays. These variables were analyzed with a completely random design with a 2 × 6 factorial arrangement and time-repeated

measurements. Factor A: type of surfactant: bacterial or pine oil; Factor B: emulsified oil concentration: 0, 30000, 40000, 50000, 60000 or 70000 ppm. Each treatment was performed in triplicate. The results were highly significant (p<0.0001) for the main effects, i.e., the concentrations of emulsified oil and surfactant, and for the treatment–time interaction. For the 30000 ppm petroleum concentration, the percentages of oil removed by the bacterial surfactant and the pine oil were 93.98% and 81.39%, respectively. All *R. mangle* plants survived the bacterial surfactant treatment, in contrast to the pine oil surfactant, which caused the death of all the plants at the end of the treatment.

Keywords: Oil spill, *Rhizophora mangle*, biosurfactant, soil restoration, *Azospirillum lipoferum*

RESUMEN

El petróleo derramado en costas y estuarios se adhiere a las raíces de los árboles y plántulas, provocando su asfixia y muerte en pocos días. Para mitigar este problema se evaluó la restauración de suelos de manglar contaminados con petróleo emulsionado con un biosurfactante producido por *Azospirillum lipoferum* y con otro obtenido a partir de aceite de pino. Los propágulos de *Risophora mangle* fueron recolectados en un manglar de la comunidad El Bellote, Paraíso, Tabasco, México, y plantados en un suelo artificial Solonchack arenoso. Cuando las plantas de *R. mangle* alcanzaron tres meses de edad, su suelo se contaminó con petróleo emulsionado. Durante el experimento se evaluó el contenido de la fracción pesada de petróleo y el crecimiento bacteriano. Estas variables fueron analizadas mediante un diseño experimental completamente al azar con un arreglo factorial 2 x 6 y medidas repetidas en el tiempo. Factor A: tipo de surfactante: bacteriano y aceite de pino, factor B: concentración de petróleo emulsionado: 0, 30000, 40000, 50000, 60000 and 70000 ppm. Cada tratamiento se realizó por triplicado. Los resultados mostraron ser altamente significativos (p<0.0001) para los efectos principales: concentración de petróleo emulsionado y surfactante, así como para la interacción tiempo-tratamiento. Para 30000 ppm de petróleo emulsionado, la remoción de petróleo con surfactante bacteriano y aceite de pino fue de 93.98% y 81.39%, respectivamente. Todas las plantas de *R. mangle* sobrevivieron al tratamiento con surfactante bacterial, en contraste, el surfactante de aceite de pino provocó la muerte de todas las plantas al final del tratamiento.

Palabras claves: Derrame de petróleo, *Rhizophora mangle*, biosurfactante, restauración de suelos, *Azospirillum lipoferum*

1. INTRODUCTION

Mangrove ecosystems are composed of halo-tolerant trees or shrubs that are capable of growing in marine, estuarine, and, in some cases, freshwater environments (dos Santos *et al.*, 2021). They are in the transitional zone between terrestrial and marine ecosystems in tropical and subtropical latitudes around the world, and they are species that are highly adapted to harsh environments: variable flooding, high temperature, high sedimentation, and anoxic and salinity stress (Cuny *et al.*, 2020). The salinity levels are variable throughout the year and depend on rainwater runoff (Wadja and Anthelme, 2021). Mangroves are

recognized globally as important ecosystems for their natural wealth and for the environmental services that they provide to human populations (Duke and Schmitt, 2015; Ellison *et al.*, 2020; dos Santos *et al.*, 2021), as well as for the important social, ecological and economic roles that they play (Bandaranayake, 1998; Lahjie, 2019).

According to Bunting *et al* (2018), the total area of mangrove forests in the world is approximately 137,760 km^2, representing no more than 1% of the world's forested land (Nyanga, 2020). Mexico has the fourth largest mangrove area in the world, and together with Indonesia, Brazil, Nigeria, Australia and Malaysia, it contains approximately 51% of the world's mangrove area (Duke, 2016; Bunting *et al.*, 2018; Velázquez-Salazar *et al.*, 2021). Mangrove forests constitute unique ecosystems that are among the most productive ecosystems in the world and have biomass levels similar to those observed in tropical rainforests (Donato *et al.*, 2011; Abdelwahab *et al.*, 2019). The ecological importance lies in their roles in stabilizing and protecting coastlines; providing breeding areas; providing food and habitats for numerous species of fish, crustaceans, mollusks, insects, mammals, and reptiles; and providing nesting sites for coastal birds (Thomas *et al.*, 2017; Cuny *et al.*, 2020).

Like any other ecosystem, mangroves are vulnerable to pollution processes, particularly oil spills, which result in extensive environmental and health damage, when they are located near oil facilities or harbors or when oil tanker accidents occur (Filgueiras *et al.*, 2021). Although these ecosystems act as natural protection barriers in coastal zones and constitute valuable biological resources that provide a wide range of services, these ecosystems are undervalued, reflecting the current state of these natural habitats, with alterations, damage and disappearance. These problems are associated not only with the poor management of coastal ecosystems but also with pollution from oil spills (Richards and Friess, 2016; Machado *et al.*, 2019; Truskewycz *et al.*, 2019; Ferraz and Barrella, 2021; Filgueiras *et al.*, 2021). Anthropogenic activities and natural factors also have harmful effects on mangrove forests (Lahjie, 2019; Mathieu and Gnagbo, 2021; dos Santos *et al.*, 2021).

The state of Tabasco, located in southeastern Mexico, has made great contributions to the national economy because of its oil wealth. However, poor management of oil and its derivatives has damaged a considerable portion of the soil and contaminated water in coastal areas where mangrove forests are dominated by four species: red mangroves (*Rhizophora mangle*), white mangroves (*Laguncularia racemosa*), black mangroves (*Avicennia germinans*) and buttonwood mangroves (*Conocarpus erectus*) (Domínguez-Domínguez *et al.*, 2011). In Tabasco, mangroves are located mainly on the coast of the Gulf of Mexico and cover 201 km of the coastline, with an area of approximately 49225 hectares (Velázquez-Salazar *et al.*, 2021). For many years, there has been intense oil activity on the coasts of the states of Tabasco and Campeche, with the consequent risk of accidents that could cause severe environmental impacts on soils, rivers, wetlands and mangrove forests.

Among the several sources of contaminants that mangroves can face, oil spills are the most worrisome because of the magnitude of the environmental disasters they can cause, not only to the fauna that inhabit these ecosystems but also to the mangrove trees. The oil remains for long periods, even after mangrove forests have disappeared (Sánchez-Arias *et al.*, 2013; Machado *et al.*, 2019). As living species, mangrove trees are subject to possible mutations and morphological alterations due to contamination with chemical agents with

mutagenic activities, such as polyaromatic petroleum compounds (Filgueiras *et al.*, 2021). Requena *et al.* (2012) noted that the morphological development of the species, *R. mangle* and *A. germinans,* is altered due to oil contamination. Similarly, Proffitt and Travis (2005) reported albino mutations in red mangrove trees in historically contaminated forests in Tampa Bay, Florida, USA.

When oil is released into coastal and estuarine waters, it is deposited on the fine roots of vegetation and is adsorbed by plants. Seedlings mostly die within a few days after contact with oil. In contrast, when only their roots and sediments are covered with oil, adult trees and taller shrubs may persist for six or more months before dying. Oil-contaminated sediments cause seedling mortality, and they are smothered and killed by spills, with lighter hydrocarbons being considered the most toxic (Duke and Burns, 1999; 2000).
Restoring a severely damaged mangrove forests can take more than 25 years; in many cases, the resulting losses are comprehensive and irreversible (Duke, 2016). Dominguez-Domínguez *et al.* (2011) found at least 13 sites in Tabasco with mangrove vegetation that was contaminated by oil from oil fields, exceeding the maximum allowable limit of petroleum hydrocarbons in its heavy fraction, established by NOM-138-SEMARNAT/SSA1-2012 (SEMARNAT, 2012) and affecting the reproduction and growth of mangrove seedlings.

Given the importance of mangrove ecosystems, the objective of this research was to evaluate the restoration of contaminated mangrove soil with emulsified oil with two surfactants, one synthesized by an *Azospirillum lipoferum* strain and the other based on pine oil, as well as to observe the survival of *R. mangle* plants in an artificial oil spill under greenhouse conditions. The main variables measured during the research were the residual content of the heavy oil fraction and the number of colony-forming units.

2. MATERIALS AND METHODS

2.1. Collecting and planting of *R. mangle* propagules

Propagules of *R. mangle* were collected in the fall season during field trips according to the guidelines of Teutli-Hernández et al. (2021) in a mangrove forest located in the community of El Bellote, Paraíso, Tabasco (18°24'11.8" N and 93°11'00.5" W). After harvesting, propagules were selected on the basis of their length (22 ± 2 cm) and diameter (13 ± 2 mm) and on the basis of an absence of damage caused by predators and/or diseases that may affect their survival and growth, ensuring homogeneity among the seedlings, which had been previously rinsed, disinfected and planted in polyethylene bags of 10 cm × 25 cm with 1 kg of artificial substrate and transported to a greenhouse of the postgraduate college, Campus Tabasco. The growing seedlings were irrigated with fresh water (chlorine-free water, pH = 7.02 and electrical conductivity of 0.69 $mS.cm^{-1}$) every third day for three months until the plants were cultivated.

2.2. Soil collection and characterization

The soils used in this bioassay were collected at two different sites; the first is located in the community of El Bellote, Paraíso, Tabasco (18°24'11.8" N and 93°11'00.5" W) in an area

with abundant mangrove trees that grow in solonchak hypersaline gleyic soil (Domínguez-Domínguez *et al.*, 2011), which is the same location at which the propagules were collected. The second site is located amid well-drained coastal dunes northwest of Paraíso, Tabasco (18°25'38.8" N and 93°12'50.6" W), where the soil is sandy-sol (Zavala-Cruz *et al.*, 2009). Both soils were dried in the shade for five days. To generate an artificial substrate suitable for the growth of *R. mangle* seedlings, the two soils were mixed at a 1:1 ratio until a homogeneous mixture was obtained.

The soil characteristics included the pH, electrical conductivity, and density, as well as the organic matter content, texture, and total hydrocarbons in the heavy oil fraction. Microbiological and elemental composition analyses were also conducted. For physical–chemical characterization, the following parameters were measured according to the Mexican standard, NOM-021-RECNACT-2000 (SEMARNAT 2002): pH (Method AS-02), electrical conductivity (Method AS-18), density (Method AS-04), and organic matter content (Method AS-07). The texture of the substrate was determined by the standard granulometry method by sieving fine and coarse aggregates. The texture of the substrate was determined by the standard grain size method, in which fine and coarse aggregates were sieved via the methodology established in ASTM C 136-01 (2001). A dry aggregate of known mass was separated through a series of sieves with progressively smaller openings for determination of the particle size distribution. Each sieve retains particles larger than its openings, thus allowing the separation and classification of the substrate. Finally, each fraction of separated material was weighed to calculate the corresponding percentages and determine the particle size distribution of the substrate. The total hydrocarbon content of the heavy oil fraction was measured according to the procedure established in the Mexican Standard NMX-AA-134-SCFI-2006 (SEMARNAT, 2012a). Biological parameters: The viable cell count, expressed as CFU/g dry soil, was obtained using the pour plate method from serial dilutions (Madigan et al. 2017).

For morphological analysis, the samples were deposited on double-sided carbon conductive tape on an aluminum sample holder. The samples were then observed with a JEOL JSM-6010LA scanning electron microscope (SEM) at an accelerating voltage of 20 kV under high vacuum conditions at different magnifications. An energy dispersive detector (EDS) coupled with the SEM was used to perform semiquantitative and chemical element distribution analyses on the surfaces of the samples. The images were processed in InTouchScopeTM software.

2.3. Bacterial broth production

A. lipoferum, which is classified as a petrophilic and biosurfactant producer (Ojeda-Morales *et al.*, 2016), was used as the bacterial strain for the bioassay. This strain was registered under the Forestry Laboratory of the Postgraduate College, Campus Tabasco, Mexico. The bacterial strain was reactivated under axenic conditions (Madigan *et al.*, 2017) and cultivated in the presence of Congo red as an indicator of the presence of nitrogen-fixing bacteria.

To stimulate the production of surfactants, the culture medium proposed by Kim *et al.* (2000), consisting of yeast extract (0.2 g), meat extract (5 g), glucose (2 g), KH_2PO_4 (0.2 g), K_2HPO_4 (0.3 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), Na_2SO_4 (0.1 g), $CaCl_2$ (0.01 g), $FeSO_4$ (0.01 g), and distilled H_2O (1 L), was used. The bacterial inoculum was adjusted to a concentration of 6.8×10^8 CFU/mL and incubated for 72 hours, according to Ojeda-Morales *et al.*, (2016), to achieve the highest surfactant production. Surfactant production was carried out in three 20-L glass

containers, with each one including an assembly in the cap to provide a sterile air supply, which was supplied by a compressor connected to a membrane filter at 28 ± 2°C and atmospheric pressure.

2.4. Experimental design

The proposed experimental design was completely randomized with the following 2 × 6 factorial arrangement: Factor A: 2 levels: biological surfactant and commercial pine oil-based surfactant; and Factor B: 6 levels of petroleum concentrations: C1 (0 ppm), C2 (30000 ppm), C3 (40000 ppm), C4 (50000 ppm), C5 (60000 ppm) and C6 (70000 ppm). Each treatment consisted of 36 experimental units, of which three repetitions of each treatment were evaluated monthly over 11 months.

2.5. Establishment of the bioassay

As the adverse effects of oil spilled on *R. mangle* plants under 1 year of age can be addressed with emulsification (Duke *et al.*, 2000; Duke, 2016), in this bioassay, an emulsion of the oil with unpurified broth was used due to the petrophilic nature of the inoculated strain. Isthmus-type oil (°API = 31.97) was used. In addition, another pine oil-based surfactant (commercial product, 30% concentration, purchased from EcoChem, Mexico) was used to compare the effects of both surfactants on the *R. mangle* plants.

Oil and surfactant emulsions were prepared according to the oil concentrations established in the experimental design and with different amounts of surfactant depending on the emulsion capacity of each surfactant. The mixtures were homogenized using a mechanical stirrer at 3,000 rpm for 3 minutes and then added to the corresponding treatments. The soil of *R. mangle* plants was contaminated with emulsified petroleum with bacterial and pine surfactants when the plants were 3 months old. The plants were irrigated every third day until the eleventh month of the experiment.

2.6. Analysis of contaminated soil during the experiment

Soils with *R. mangle* plants that were contaminated with emulsified oils containing bacterial surfactants and pine oil were evaluated every month for 11 months. The response variables were defined as the heavy oil fraction (HOF) and number of colony-forming units (CFU/g dry soil). During the bioassay evaluation period, the chemical composition of the substrate was also monitored every two months to determine the elemental chemical composition of the soil and to identify chemical elements that could be indicators of contamination; the analyses were carried out with a JEOL scanning electron microscope (model JSM-6010LA). The sodium chloride concentration was quantified according to the following procedure: A 10-g sample of soil was added to 20 mL of distilled water, stirred on a vortex stirrer for 15 minutes and left to stand for 15 minutes. Finally, the NaCl concentration of the supernatant was determined using a model HI98319 salinity meter (Hanna Instruments). The concentration of the heavy oil fraction was determined by the gravimetric method. Hydrocarbon extraction was performed with a Soxhlet extractor with *n*-hexane as the solvent (Aldrich). The extract was recovered by filtration and subjected to distillation to separate the solvent. The amount,

in g, of the extracted material was determined by gravimetry. The procedure followed is established in the Mexican Standard, NMX-AA-134-SCFI-2006 (SEMARNAT, 2012a).

2.7. Statistical analysis

The response variables were analyzed with a completely randomized design with a factorial arrangement of 2 × 6 treatments and repeated measurements over time using the general linear model (GLM) procedure and the PROC MIXED function of SAS (SAS Inst. Inc., 2004). Each treatment combination (surfactant type × concentration) consisted of three repetitions with monthly measurements. The data were expressed as the means of least squares ± EEM (standard error) and compared to the results of the adjusted Tukey test (Herrera-Haro et al., 2010).

3. RESULTS

3.1. Initial soil characterization

The results of the initial characterization of the artificial soil, which was composed of a mixture of mangrove and dune soils, are shown in Table 1. Because a bioassay was established to assess the degradation of the oil contained in each treatment, oil contaminants were determined before the establishment of the bioassay. No hydrocarbons corresponding to the heavy fractions were detected in the soil used, so the initial concentrations that were measured at the beginning of the assessment were those added in each treatment. On the other hand, within the physicochemical characterization, an approximately neutral pH (7.2) was determined for the soil, with a low electrical conductivity level (1.13 mS/cm) corresponding to slightly saline soils. A value of 1.2 g/mL was obtained for the density that corresponded to loamy soils. This corroborates the results of the granulometric analysis, which revealed that the soils were composed mainly of sand (58.9%), followed by silt (41%) and very little clay (0.1%). According to this particle size distribution, the textural class of the substrate is sandy loam. These characteristics corroborate the result of the organic matter determination of 1.34%, which corresponds to the classification of mineral soils (0.6–1.5%) (Gasch and DeJong-Hughes, 2019). The mineral-dominant nature of the substrate is also supported by the results of the elemental composition analysis that was performed by SEM, where the distribution of elements was found to be (mass %) carbon 15.31, nitrogen 0.84, oxygen 59.57, sodium 1.07, magnesium 1.32, aluminum 5.1, silicon 13.5, phosphorus 0.08, potassium 0.73, calcium 0.65 and titanium 0.17 and smaller amounts of Fe, Pb and V. On the other hand, the microbiological analysis of the soil revealed a relatively low estimated value of bacteria that was equivalent to $1.13x10^5$ CFU/g dry soil.

3.2. Degradation of heavy oil fraction (HOF)

There was a decrease in the HOF concentration during the 11-month assessment period. The decreases in oil concentration that were recorded in each bacterial surfactant treatment were as follows (ppm): 30000 to 1806, 40000 to 2825, 50000 to 6075, 60000 to 7906 and 70000 to 12148 (Fig. 1). The highest oil degradation rates corresponded to the lowest initial oil concentrations. At higher initial concentrations, lower degradation rates were observed,

which is consistent with the decreases in bacterial populations that were found at these oil concentrations; high concentrations are likely toxic to microorganisms (Hernández *et al.*, 2011).

Table 1. Physicochemical and biological initial characterization of the artificial soil employed in bioassays.

Soil Characterization								
Hydrocarbons	Physicochemical characteristics				Granulometric analysis			Microbial density
HOF [a] (mg/kg)	pH	EC [b] (mS/cm)	Density (g/mL)	OM [c] (%)	Sand (%)	Silt (%)	Clay (%)	(CFU/g dry soil)
NF[d]	7.2	1.13	1.2	1.34	58.9	41	0.1	1.13×10^5

[a] heavy oil fraction, [b] electrical conductivity, [c] organic matter, [d] not found.

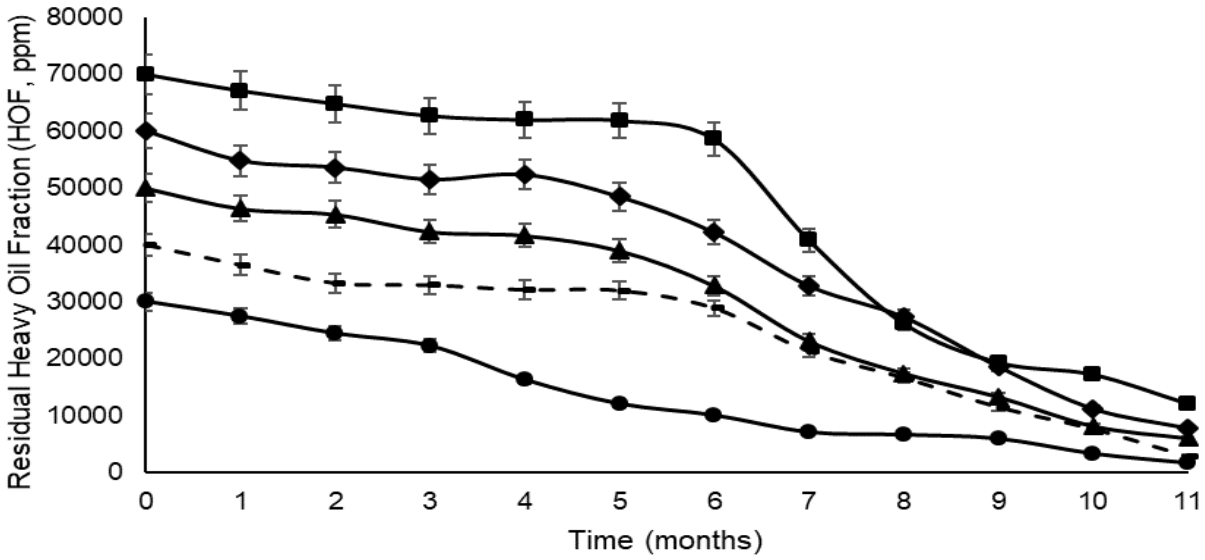

Fig. 1. Oil concentration through the time of bioassay evaluation. Emulsified petroleum concentration with bacterial surfactant (ppm): —●— 30000, - - - 40000, —▲— 50000, —◆— 60000, —■— 70000.

The analysis of repeated measurements for the variable heavy oil fraction in ppm of petroleum produced a significant result for the interaction of the emulsified petroleum

concentration with the bacterial surfactant per time (p<0.0001). These findings indicate that both factors influence the removal of emulsified oils.

When the results that were obtained with the bacterial surfactant emulsion were compared with those obtained with the pine oil surfactant, even with the lowest level of oil removal, the rate with the bacterial-based surfactant was higher than that achieved with the oil that was emulsified with the pine oil-based surfactant. The percentages of residual HOF that were measured at the end of the bioassay revealed that the treatments with oil emulsified with bacterial surfactant presented greater removal rates of HOF, reaching 93.98% for the treatment at 30000 ppm (Fig. 2). At the same oil concentration, the percentage in the treatments with emulsified oil with pine oil surfactant reached 81.03%. At this petroleum concentration, the removal of the emulsified oil by the bacterial surfactant was approximately 12.95% greater than that by the pine oil surfactant. On the other hand, as the oil concentration increased, the pine oil surfactant was shown to be less effective in removing hydrocarbons from the heavy fraction. Thus, for the highest concentration of emulsified oil, the bacterial surfactant treatment removed 82.6% of HOF at the end of the experiment, whereas the pine oil treatment removed only 56.2%.

The percentages of oil removal were greater at lower oil concentrations and lower at higher oil concentrations. This experimental observation is consistent with the fact that the higher the concentration of oil in a contaminated soil is, the lower the level of oxygenation due to the hydrophobic coating provided by the oil. The importance of oxygen in the soil comes from the participation of oxygenases and molecular oxygen in the main hydrocarbon degradation pathways (Madigan *et al.*, 2017).

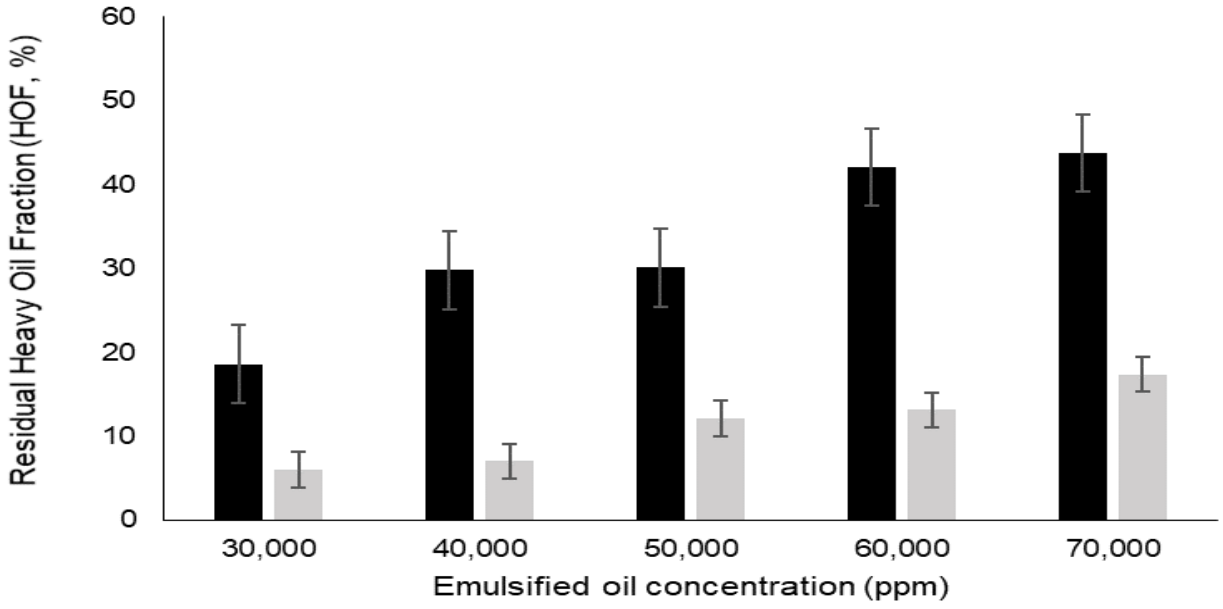

Fig. 2. Effect of the concentrations of oil emulsified with bacterial ■ and pine surfactants ■, on the residual of heavy oil fraction quantity in contaminated soil tested, at the end of the experiment.

3.3. Evaluation of colony-forming units (CFU/g dry soil)

The addition of oil emulsified with bacterial surfactant to the bioassays induced a bioaugmentation process because the bacterial surfactant was added without separating it from the culture medium and biomass. High amounts of CFU/g dry soil were measured in the treatments at 30000 ppm ($\overline{x}$ 117262 ± 8360.5), 40000 ppm ($\overline{x}$ 132059 ± 5728.1) and 50000 ppm ($\overline{x}$ 120114.6 ± 5332.3) immediately after contamination (Fig. 3).

In the middle of the treatment, the dry soil levels in CFU/g dropped, (Fig. 3). There was a 90% decrease in the 40000 ppm treatment group and a 98% decrease in the 60000 ppm treatment group. The highest population density corresponded, for the most part, to heterotrophic microorganisms in general and not to hydrocarbon-degrading microorganisms. Thus, when readily biodegradable organic substrates were consumed, the number of CFUs per g of dry soil gradually decreased during the first five months of treatment. After 6–8 months of treatment, a drastic reduction in the bacterial populations was subsequently observed, probably due to the depletion of shorter-chain hydrocarbons. At this stage, perhaps only petrophilic bacteria capable of degrading longer-chain hydrocarbons survived, generating more bioavailable byproducts by the end of the eighth month. From the ninth month of treatment to the end of the experiment, a slight increase in the bacterial populations was observed, probably due to their ability to adapt to and degrade longer-chain hydrocarbons.

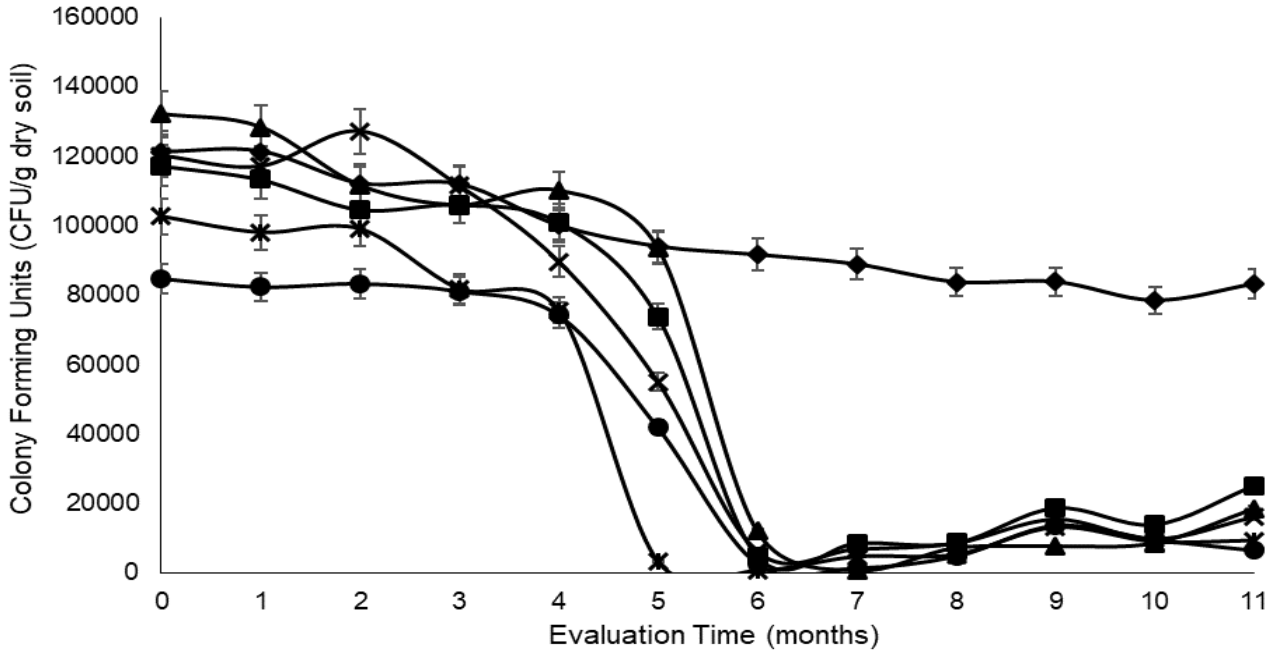

Fig. 3. Effect of emulsified oil concentrations on colony forming units (CFU/g dry soil) during 11 months of evaluation with petroleum emulsified with bacterial surfactant. Concentration of emulsified petroleum (ppm): 0, 30000, 40000, 50000, 60000, 70000.

The analysis of the repeated measurements of the number of colony-forming units (CFU/g dry soil) produced a significant result for the effect of the interaction of the emulsified oil concentration per time (p<0.0001). These findings indicated that both factors influenced microbial population growth.

A drastic decrease in the bacterial populations was observed in all the treatments, especially in the treatment with oil emulsified with pine oil surfactant, which was probably due to the toxicity of this oil (Politeo *et al.*, 2011). In the case of the control treatment with a bacterial surfactant, there was a gradual decrease in the bacterial population, which at the end of the treatment resulted in a decrease of 31% with respect to the initial population; this result was probably due to the scarcity of nutrients. As the concentration of emulsified oil increased, the bacterial population decreased in each treatment, regardless of the surfactant used; i.e., greater concentrations of emulsified oil exhibited greater toxicity to microorganisms (Fig. 4). At the end of the experiment, the microbial populations in the pine oil surfactant control group showed greater decreases than those in the bacterial surfactant control group did, reflecting the toxicity of the pine oil surfactant toward microorganisms.

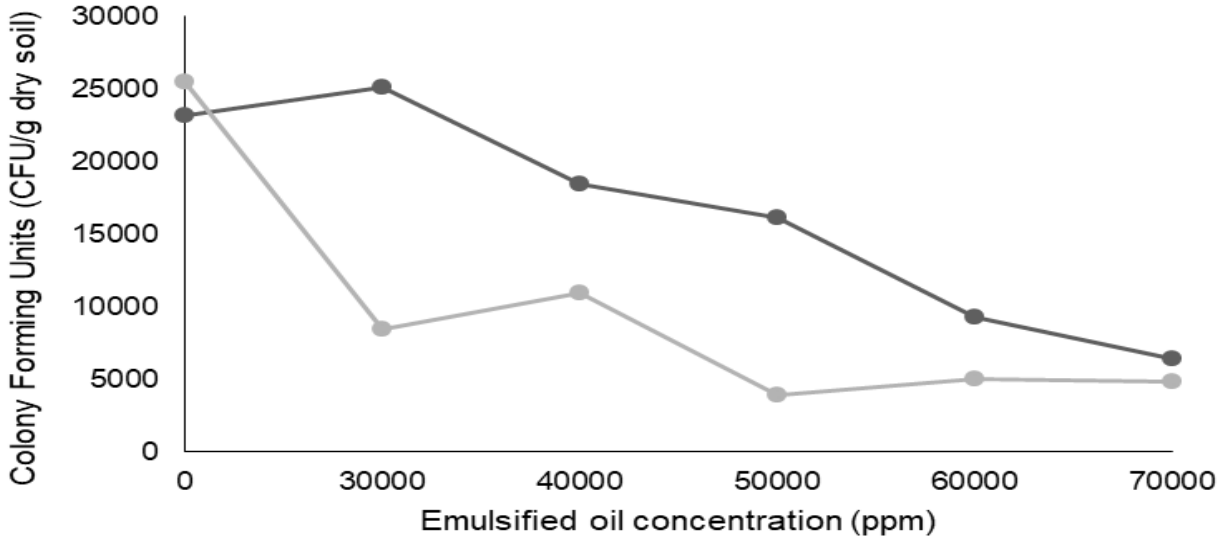

Fig. 4. Effect of oil concentrations in emulsions with bacterial and pine surfactants on CFU/g dry soil in contaminated soils, at the end of bioassays: bacterial surfactant, pine surfactant

4. DISCUSSION

The parameters that were obtained from the substrate characterization, namely, a pH of 7.2 and organic matter content of 1.34%, are consistent with those reported by other researchers for similar substrate mixtures (Dominguez-Dominguez *et al.*, 2011; Abdelwahab *et al.*, 2019). However, Verane *et al.* (2020) reported that in sandy sediment, the organic

matter content was 0.10%. In terms of the pH, according to Semboung *et al.* (2016), most microorganisms grow at pH values ranging from 5.0-9.0, with optimal values near neutral, as in this research. pH affects microorganisms and microbial enzymes and determines the CO_2 solubility and the availability of nutrients such as ammonium and phosphates.
The values of the abovementioned parameters agree with those reported for the soils that were used in the substrate mixture, since the organic matter content of both soils averaged 1.34%, while the pH was 7.2. On the other hand, the greatest variation was found for the electrical conductivity, since the mangrove soil (hypersaline gleyic solonchak) had a value of 117.9 mS/cm; however, when the final texture of the substrate and the predominance of sand were considered, the electrical conductivity had a value of 1.13 mS/cm, which is slightly higher than the value of 0.03 mS/cm that has been previously reported for dune soils (sandy-sol eutric soil) (Zavala-Cruz *et al.*, 2009).

Soil microbiology shows that the population of microorganisms is one of the parameters that is used to establish quality standards for soil because these organisms are sensitive to the changes generated by their use (Sarto *et al.*, 2020; Bobul'ská *et al.*, 2021). The number and variety of soil microorganisms are also related to the presence of biological or anthropogenic disturbances to which the soil has been exposed (López-Jiménez *et al.*, 2019; Iturbe-Espinoza *et al.*, 2022). According to Begum (2020), the average number of bacteria found in mangrove soils is also a function of soil porosity, soil organic matter and sediment depth, which are greater in the surface layers and decrease with depth and are also a function of environmental factors such as soil pH and nutrients. The bacterial abundance was estimated to be $1.13x10^5$ CFU/g dry soil, which is lower than that reported by several researchers for bacteria isolated from the roots of mangrove trees (Genthner *et al.*, 2013; Ramírez-Elías *et al.*, 2014; Fakhrzadegan *et al.*, 2019), resulting in a relatively low value because, in this research, a mixture of mangrove soil and dune soil was used.

The observed hydrocarbon degradation rates differed between the two surfactants used, with the highest degradation rates observed in the bioassays with oil emulsified with the bacterial surfactant. Owing to the reduction in surface and interfacial tension, the addition of surfactants can cause the desorption of oil from the soil, leading to increased bioavailability of hydrocarbon compounds and higher degradation rates. In a previous report, the bacterium *A. lipoferum* was identified as a biosurfactant producer (Ojeda-Morales *et al.*, 2015).
In this context, Cortés-Sánchez *et al.* (2013) noted that the application of oil emulsified with bacterial surfactants reduces its negative effects on seedlings, increasing wetting. The surfactant produced by *A. lipoferum* contains lipopeptides, which are low-molecular-weight compounds and possess excellent emulsifying, dispersing and wetting properties with the ability to reduce surface tension (Ojeda-Morales *et al.*, 2016).

One important factor that affects the restoration of oil-contaminated soils is the bioavailability of contaminants. Surfactants can be applied to increase the desorption and dissolution of hydrophobic organic contaminants in soils and sediments and, as a result, improve the biodegradation process. Surfactants facilitate mass transfer, helping make low-solubility carbon sources bioavailable for biodegradation and introduction into the different metabolic pathways for energy generation and biomass synthesis. Through this process, which occurs over several stages, the degradation of oil, an increase in the bacterial populations and the growth of *R. mangle* plants occur. On the other hand, in microbial surfactants, in addition to

compounds with surfactant activity, other metabolites with solvent activity are also present (Duke, 2016).

Furthermore, *R. mangle* itself efficiently promotes the hydrocarbon degradation process with the help of enzymes, hormones and nutrients released by its roots, which modify the soil composition near the roots and stimulate the growth of the associated microbiota (Verane *et al.*, 2020), which uses plant exudates such as carbohydrates and organic acids that are easy for bacteria to use. This labile organic matter stimulates the growth of microorganisms and their activities, resulting in the biodegradation of organic contaminants (dos Santos *et al.*, 2021). Thus, the presence of mangrove vegetation may accelerate the restoration process (Verane *et al.*, 2020).

The removal of oil from soil or sediment with mangrove vegetation occurs through phytotransformation and rhizodegradation, which are important processes associated with phytoremediation. Microbial degradation in the rhizosphere can be the primary mechanism for cleaning a variety of oil-contaminated soils, including the removal of oil-containing contaminants in water by aquatic plants (Rivera-Cruz *et al.*, 2016) and in mangrove sediments. However, petroleum hydrocarbons are considered highly hydrophobic compounds. The success of rhizodegradation depends on the interactions between specific microorganisms, appropriate environmental conditions and nutrient availability (Moreira *et al.*, 2011).

The lower the oil concentration was, the higher the degradation rate was. In research on crude oil-degrading bacteria from mangrove ecosystems, Fakhrzadegan *et al.* (2019) reported that the optimum crude oil concentration was 4% by weight (*i.e.,* 40000 ppm). Our results revealed that degradation was efficient even at relatively high concentrations; however, at concentrations above 60000 ppm, the degradation rate was relatively low, which can be attributed to the toxicity to microorganisms and mangrove plants caused by high oil concentrations (Semboung *et al.*, 2016). Nevertheless, the level of hydrocarbon oil toxicity depends on the mangrove species (Lewis *et al.*, 2011; Cuny *et al.*, 2020).

On the other hand, at the same petroleum concentration, the lowest degradation rates were observed with the emulsion of petroleum and pine oil, which is a complex mixture of organic compounds. The qualitative and quantitative composition of the essential oils of conifers depends on the species, its territorial origin and the part of the plant analyzed (Ismail *et al.*, 2013).

The toxic effect of pine oil may be due to its composition, as it is composed of a mixture of monoterpenes and sesquiterpenes with variable amounts of *α*-pinene, ranging from low (0.41%) to high concentrations (82.9%), depending on the species of pine tree and geographical location (Tahar *et al.*, 2005; Zeng *et al.*, 2012; Ismail *et al.*, 2013; Abd-ElGawad *et al.*, 2021). *α*-pinene is considered the most abundant and chemically exploited monoterpene by industry because it is accessible and inexpensive (Liu, 1999) and serves as a building block for other chemicals with many applications (Allenspach and Steuer, 2021). Owing to the toxicity of pine oil, when the surface-active emulsion of pine oil was poured on the substrate, its main effect may have contributed to the deactivation of the characteristics of the microbiota of the plant's rhizosphere (Politeo *et al.*, 2011), thus

preventing the biostabilizing and remedial actions that were performed by the microorganisms present from assisting in the transformation of the spilled oil.
On the other hand, the bacterial population density expressed as CFU/g dry soil was greater in the treatments with oil emulsified with the bacterial surfactant than in the treatments with the pine oil emulsion. In the treatment with 30000 ppm emulsified oil with bacterial surfactant, at the end of the experiment, the bacterial population was 2.5 x 10^4 CFU/g dry soil, whereas in the treatment with pine oil emulsion, it was 8.4 x 10^3 CFU/g dry soil. The treatments with the pine oil surfactant caused 100% death of the *R. mangle* plants; however, all the *R. mangle* plants that were treated with oil emulsified with the bacterial surfactant survived the treatment. Therefore, according to Moreira *et al.* (2011), there may no longer be a partnership between the plant rhizosphere and the microorganism community, as reflected by the decline in the bacterial population.

In this work, the bacterial population density of the treatments with emulsified petroleum with pine oil was reduced, mainly for the treatments with relatively high oil concentrations. At the end of the experiment, the pine oil-based surfactant was effective at dispersing the oil, but it could have been toxic to *R. mangle* plants and rhizospheric microorganisms due to its chemical nature.

As expected, the HOF concentrations decreased over time in both bioassays. The highest oil removal level of 93.98% was achieved in the treatment with 30000 ppm oil emulsified with bacterial surfactant, whereas the lowest removal rate of 56% was achieved in the treatment with 70000 ppm and pine oil as the emulsifying agent. The magnitude of oil removal may be related to the bacterial population density of the soil substrates and the presence or absence of *R. mangle* plants, which may have favored the growth of bacteria (Moreira *et al.*, 2011; Verane *et al.*, 2020). In the treatment with 30000 ppm oil emulsified with bacterial surfactant, the bacterial population density was 73.3% greater than that in the treatment with 70000 ppm oil emulsified with the same surfactant. The higher the oil concentration is, the greater the toxicity, which can cause inadequate conditions for petrophilic microorganisms that carry out the bioremediation process.

At the end of 11 months of treatment and evaluation, the elemental analysis of the oil-contaminated soil provided interesting data that were most clearly observed for the treatment with 30000 ppm emulsified oil. The greatest growth of *R. mangle* plants and oil removal were recorded in this treatment, and the soil elements determined were C (10.8%) and O (1.57%), demonstrating a decrease with respect to the initial analysis. Decreases in other elements, such as Na (-82.22%), Mg (-44.94%), Al (-62.13%), Si (-15.72%), K (-27.45%), Ca (-87.97%), Fe (-14%), Pb (-0.73%) and V (-1.46%), were observed. Titanium was not detected in the final analysis. Elements such as Na, Mg, K and Ca are considered interchangeable bases, and their abundance or deficiency depends on the cation exchange capacity (CEC) of soils, which indicates the availability and quantity of nutrients available to plants.

The CEC depends on the texture of the soil (Zavala-Cruz *et al.,* 2009). For the soil used in the bioassay, a franc-sandy texture was determined, which is consistent with a low capacity to retain nutrients in addition to a high level of permeability such that the interchangeable bases are more easily leached when irrigation water is applied (Gutiérrez and Zavala-Cruz,

2002). The presence and accumulation of metals in mangrove sediments are the result of anthropogenic activities around mangrove ecosystems, such as wastewater discharge and oil industry activities (Liu *et al.*, 2015). Therefore, the presence of vanadium and lead in the soils of the bioassay may be attributed to the presence of previously acquired contaminants in addition to the spilled oil.

For all the treatments with emulsified oils with bacterial surfactants, the effects of sodium chloride were similar to those of metals; an approximately similar decrease was recorded for each treatment: 30000 ppm (-44.44%), 40000 ppm (-37.7%), 50000 ppm (-40%), 60000 ppm (-42.55.83%) and 70000 ppm (-43%). The observed decreases in NaCl concentrations in the substrates of soils with *R. mangle* vegetation contaminated with oil emulsified with biosurfactant could be attributable to soil leaching because of constant irrigation and to the ability of the mangroves to excrete salts. Most mangrove species exclude sodium chloride and other salts that are dissolved in seawater by accumulating chlorine and sodium ions in leaf vacuoles, where they remain sequestered far from the active sites of the cells (Hoff *et al.*, 2014).

5. CONCLUSION

The results obtained in this work confirm that the environmental impact caused by oil spills could be minimized when oil is emulsified with a bacterial surfactant synthesized by *A. lipoferum*. The studied solonchak-sandy soil mixture contaminated with 30000 ppm oil emulsified with bacterial surfactant achieved, at the end of the experiment, greater oil hydrocarbon removal (93.98%) and a bacterial population of 25,149 CFU/g dry soil than did the treatments with oil emulsified with pine surfactant, for which the maximum oil hydrocarbon removal rate was 81.39%, and the maximum bacterial population was 8,463 CFU/g dry soil. The oil that was emulsified with the bacterial surfactant caused the least damage to the *R. mangle* plants, in contrast to the harmful effects caused by the pine oil surfactant. The application of the oil-broth emulsion achieved the highest removal of HOF, revealing that this is a promising technique for the restoration of oil-contaminated soil with *R. mangle* vegetation. It is important to obtain dose–response data for soils affected by oil spills and to follow the long-term processes associated with oil accumulation and decomposition in sediment owing to the presence of complex plant–soil–microorganism interrelationships, and it is clear that each depends on the other; therefore, a modification to the conditions of one will affect the others.

AUTHOR CONTRIBUTIONS

M.E. Ojeda-Morales and A.L. Severo-Domínguez: experimental; M. Domínguez-Domínguez and M.A. Hernández-Rivera: conceptualization, supervision and writing; J. Zavala-Cruz and J.G. Herrera-Haro: statistical analysis; J.G. Álvarez-Ramírez and C.M. Morales-Bautista: editing and review.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Abd-ElGawad, A.M., Abd El-Nasser, G., Gendy, E.l., Abdulaziz M., Assaeed S.L., Al-Rowaily, Abdullah S., Alharthi T.A., Mohamed M.I., Yaser H.D., and Abdelsamed I.E., 2021. Phytotoxic effects of plant essential oils: A systematic review and structure-activity relationship based on chemometric analyses. Plants 10(1):36. https://doi.org/10.3390/plants10010036

Abdelwahab, A.A., Mohamed, S.A., Amira, S.S., Abdel-Hamid, A.K. and El-Bialy, E.H., 2019. Physical and chemical characteristics of mangrove soil under marine influence. A case study on the mangrove forests at Egyptian - African Red Sea coast. Egyptian Journal of Aquatic Biology and Fisheries 23. 385-399. 10.21608/ejabf.2019.47451

Allenspach, M. and Steuer, C., 2021. α-Pinene: A never-ending story. Phytochemistry Volume 190, 112857. https://doi.org/10.1016/j.phytochem.2021.112857

ASTM C 136-01., 2001. Standardized test method for determining the particle size analysis of coarse and fine aggregates. Astm C 136-01.doc [2nv8j6v79rlk] (idoc.pub). Accessed June 2024.

Bandaranayake, W., 1998. Traditional and medicinal uses of mangroves. *Mangroves and Salt Marshes* 2, 133–148. https://doi.org/10.1023/A:1009988607044

Begum, A., 2020. Variation of bacterial population in four soil depths in the sundarbans mangrove forest. Bangladesh. Int. J. Adv. Res. Biol. Sci. 7(6): 65-76. http://dx.doi.org/10.22192/ijarbs.2020.07.06.006

Bobul'ská, L., Espíndola, S.P., Coelho, M.A., Ferreira, A.S., 2021. Impact of land use on soil function and bacterial community in the Brazilian savanna. An Acad Bras Cienc. Sep 20;93 (suppl 3): e20201906. https://doi.org/10.1590/0001-3765202120201906

Bunting, P., 2018. The global mangrove watch—A new 2010 global baseline of mangrove extent remote sensing 10(10), 1669. https://doi.org/10.3390/rs10101669

Cortés-Sánchez, A., Troconis, I. and Jaramillo, M., 2013. Biological surfactants with potential application in food technology: a review. Biológicas 15(1), 16-23. https://www.biologicas.umich.mx/index.php?journal=biologicas&page=issue&op=view&path%5B%5D=12. Accessed 10 June, 2024

Cuny, P., Jezequel, R., Michaud, E., Sylvi, L., Gilbert, F., Fiard, M., Chevalier C., Morel, V. and Militon, C., 2020. Oil spill response in mangroves: why a specific ecosystem-based

management is required? The case of French Guiana – a mini-review. Vie et Milieu, 70 (3-4). 69-76. ISSN 0240-8759. https://wwwphp.obs-banyuls.fr/Viemilieu/index.php/volume-70-2020/70-issue-3-4/70-3-4-article-9/download.html

Domínguez-Domínguez, M., Zavala-Cruz, J. and Martínez-Zurimendi, P., 2011. Sustainable forest management of Tabasco's mangroves. Secretariat of Natural Resources and Environmental Protection. Postgraduate College. Villahermosa, Tabasco, Mexico. http://sostenible.palencia.uva.es/system/files/publicaciones/LIBRO_MANGLARES_DE_TABASCO.pdf. Accessed 21 July 2024

Donato, D., Kauffman, J., Murdiyarso, D. et al., 2011. Mangroves among the most carbon-rich forests in the tropics. Nature Geosci 4, 293–297. https://doi.org/10.1038/ngeo1123

Dos Santos, G., Sershen, J., and França, M.G.C., 2021. Mangrove assisted remediation and ecosystem services. In handbook of assisted and amendment: Enhanced sustainable remediation technology, M.N.V. Prasad (Ed.). https://doi.org/10.1002/9781119670391.ch26

Duke, N.C., 2016. Oil spill impacts on mangrove: Recommendations for operational planning and action based on global review. Marine pollution bulletin, 109 (2), 700-715. doi.org/10.1016/j.marpolbul.2016.06.082

Duke, N.C. and Burns, K.A., 1999. Fate and effects of oil and dispersed oil on mangrove ecosystems in Australia. Environmental implications of offshore oil and gas development in Australia: Further research. A compilation of three scientific marine studies. Australian Petroleum Production and Exploration Association (APPEA), Canberra, pp. 232–363 (521 pp.). https://www.researchgate.net/publication/37629636

Duke, N.C. and Schmitt, K., 2015. Mangroves: Unusual forests at the seas edge, in: Pancel, L. M., (Eds), tropical forest handbook. Springer-Verlag, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-41554-8_129-1

Duke, N.C., Burns, K.A., Swannell, R. P., Dalhaus, O. and Rupp, R., 2000. Dispersant use and a bioremediation strategy as alternate means of reducing impacts of large oil spills on mangroves: The Gladstone field trials. Marine pollution bulletin, 41, 403-412. https://doi.org/10.1016/S0025-326X(00)00133-8

Ellison, A.M., Felson, A.J. and Friess, D.A., 2020. Mangrove rehabilitation and restoration as experimental adaptive management. Front. Mar. Sci. 7:327. doi: 10.3389/fmars.2020.00327

Fakhrzadegan, I., Hassanshahian, M., Askari, H. M., Saadatfar, A., 2019. A study of crude oil-degrading bacteria from mangrove forests in the Persian Gulf. Mar Ecol.; 40: e12544. https://doi.org/10.1111/maec.12544

Ferraz, D.R. and Barrella, W., 2021. Scient metrics analysis of mangrove studies from 1980 to 2020. J Aquac Mar Biol., 10(4):188–192. DOI: 10.15406/jamb.2021.10.00320

Filgueiras, F., Freitas, D., Motteran, F., Soares, F.B. and Gavazza, S., 2021. Bioremediation of polycyclic aromatic hydrocarbons in contaminated mangroves: Understanding the historical and key parameter profiles. Marine Pollution Bulletin, volume 169, 112553, ISSN 0025-326X. https://doi.org/10.1016/j.marpolbul.2021.112553

Gasch, C., and DeJong-Hughes, J., 2019. Soil organic matter does matter. North Dakota State University, https://www.ndsu.edu/agriculture/sites/default/files/2022-08/sf1942.pdf. Accessed 12 July, 2024

Genthner, F.J., Lewis, M.A., Nestlerode, J.A., et al., 2013. Relationships among habitat quality and measured condition variables in Gulf of Mexico mangroves. Wetlands Ecol. Manage 21, 173–191. https://doi.org/10.1007/s11273-013-9289-3

Gutiérrez, C.M.C. and Zavala, C.J., 2002. Hydromorphic features of hydrocarbon-contaminated tropical soils with hydrocarbons. Terra Latinoamericana, Vol. 20, núm.2, pp.101-111. https://www.redalyc.org/articulo.oa?id=57320202

Hernández, R.M.A., Ojeda, M.M.E., Martínez, J., Córdova, B.Y., 2011. Optimal parameters for *in vitro* the development of the hydrocarbonoclastic microorganism *Proteus* sp. Soil Sci. Plant Nutr., 11, 29-43. doi:10.4067/S0718-95162011000100003

Hoff, R., Michel, J., Hensel, P., Proffitt, C., Delgado, P., Shigenaka, G., Yender, R., Mearns, A., 2014. Oil spills in mangroves: Planning and response considerations. Washington, D.C.: National Oceanic and Atmospheric Administration, NOAA Ocean Service, Office of Response and Restoration. https://response.restoration.noaa.gov/sites/default/files/Oil_Spill_Mangrove.pdf. Accessed 05 March, 2024

Ismail, A., Hamrouni, L., Hanana, M., Gargouri, S. and Jamoussi, B., 2013. Chemical composition, bio-herbicidal and antifungal activities of essential oils isolated from Tunisian common cypress (*Cupressus sempervirens L*.). Journal of medicinal plant research. 7. 1070-1080. doi: 10.5897/JMPR12.1088

Iturbe-Espinoza, P., Bonte, M., Gundlach, E., Brandt, B.W., Braster, M., van Spanning, R.J.M., 2022. Adaptive changes of sediment microbial communities associated with clean-up of oil spills in Nigerian mangrove forests. Marine Pollution Bulletin 176 (2022) 113406. https://doi.org/10.1016/j.marpolbul.2022.113406

Herrera-Haro, J. G., García, A. C and Enrique, S., 2010. Biostatistics in veterinary sciences: Data analysis procedures with SAS. Complutense University of Madrid, Health Sciences Area, ISBN 978-84-96704-32-9

Kim, H., Lim, E.J., Lee, S.O., Lee, T.H., 2000. Purification and characterization of biosurfactans from *Nocardia sp*. L-417. Biotechnol. Appl. Biochem, 31 (3), 249-253. https://doi.org/10.1042/BA19990111

Lahjie, A.M., Nouval, B., Lahjie, A.A., Ruslim, Y. and Kristiningrum, R., 2019. Economic valuation from direct use of mangrove forest restoration in Balikpapan Bay, East Kalimantan, Indonesia [version 2; peer review: 2 approved] F1000Research, 8:9. https://doi.org/10.12688/f1000research.17012.2

Lewis, M., Pryor, R. and Wilking, L., 2011. Fate and effects of anthropogenic chemicals in mangroves ecosystems: a review. Environ. Pollut. 159(10), 2328-2346. DOI: 10.1016/j.envpol.2011.04.027

Liu, J., Wub, H., Feng, J., Li, Z., Lin, G., 2015. Heavy metal contamination and ecological risk assessments in the sediments and zoobenthos of selected mangrove ecosystems, South China. Catena 119, 136-142. https://doi.org/10.1016/j.catena.2014.02.009

Liu, W., 1999. Terpenes: Expansion of the chiral pool. Handbook of chiral chemical. Ed. D. Ager. NSC Technologies (pp. 83-102). Illinois, USA

López-Jiménez, J.G., Rivera-Cruz, M.C., Trujillo-Narcía, A., Aceves-Navarro, L.A., Sol-Sánchez, A. and Sarracino-Martínez, O., 2019. *Laguncularia racemosa* (*Combretaceae*) and associated terrestrial bacteria half a century after chronic oil contamination. Revista de Biología Tropical 67(6) 1194-1209. https://doi.org/10.15517/rbt.v67i6.35861

Machado, L.F., de Assis, L. D.C., da Costa, R. C.T.C., Paes, J.E., Martins, E.F., Peixoto, R.S. and Rosado, A.S., 2019. Tracking mangrove oil bioremediation approaches and bacterial diversity at different depths in an *in situ* mesocosms system. Front. Microbiol 10:2107. doi: 10.3389/fmicb.2019.02107

Madigan, M., Martinko, J., Stahl, A. and Clark, D., 2017. Brock biology of microorganisms. 14th Edition Pearson Higher Education, Spain.

Mathieu, E. and Gnagbo, A., 2021. Status and perspectives of mangrove management in Côte d'Ivoire. GSC Advanced Research and Reviews 9 045–050. 10.30574/gscarr.2021.9.2.0252

Moreira, I., Oliveira, O., Triguis, J., Dos Santos, A., Queiroza, A., Martins, C., Silva, C., Jesus, R., 2011. Phytoremediation using *Rizophora mangle L*. in mangrove sediments contaminated by persistent total petroleum hydrocarbons (TPH's). Microchemical Journal 99(2),376–382. http://dx.doi.org/10.1016/j.microc.2011.06.011

Nyanga, C., 2020. The role of mangroves forests in decarbonizing the atmosphere. In M. Bartoli, M. Frediani and L. Rosi (Eds) carbon-based material for environmental protection and remediation. IntechOpen. https://doi.org/10.5772/intechopen.92249

Ojeda-Morales, M.E., Domínguez-Domínguez, M., Hernández-Rivera, M.A., Álvarez-Ramírez, J.G., 2016. Biosurfactant synthesized by *Azospirillum lipoferum* ALM1B2. Characterization and application for environmental protection. Water, Air & Soil Pollut. 227, 181. doi: 10.1007/s11270-016-2871-6

Ojeda-Morales, M.E., Domínguez-Domínguez, M., Hernández-Rivera, M.A., Zavala-Cruz, J., 2015. Biosurfactants production by strains of *Azospirillum* isolated from petroleum contaminated sites. Water, Air & Soil Pollut. 226, 401. doi: 10.1007/s11270-015-2659-0

Politeo, O., Skocibusic, M., Maravic, A., Ruscic, M. and Milos, M., 2011. Chemical composition and antimicrobial activity of the essential oil of endemic dalmatian black pine (*Pinus nigra ssp*. *dalmatica*). Chemistry & Biodiversity 8: 540-547. https://doi.org/10.1002/cbdv.201000185

Proffitt, C.E. and Travis, S.E., 2005. Albino mutation rates in mangroves (*Rhizophora Mangle L*.) as a bioassay of contamination history in Tampa Bay, Florida, USA. Wetlands Vol. 25, No. 2, pp. 326–334. https://doi.org/10.1672/9

Ramírez, E.M.A., Ronald, F.C., Alejandro, A., Juan, J.A., Gustavo, R.V., Luz, E.B., Fernando, J.E.G., Oscar, G.B., 2014. Identification of culturable microbial functional groups isolated from the rhizosphere of four species of mangroves and their biotechnological potential. Applied Soil Ecology Volume 82, Pages 1-10. ISSN 0929-1393. https://doi.org/10.1016/j.apsoil.2014.05.001

Requena, G., Agraz, C., Vázquez, A., Osti, J., Reyes, J., Chan, C., García, C., 2012. Evaluation of the environmental impact on mangrove seedling morphometry by crude oil. Ra Ximhai 8(2),99-105. http://www.redalyc.org/articulo.oa?id=46123333009

Richards, D.R. and Friess, D.A., 2016. Rates and drivers of mangrove deforestation in Southeast Asia 2000-2012. PNAS 113(2), 344-349. https://doi.org/10.1073/pnas.1510272113

Rivera-Cruz, M.C., Trujillo-Narcia. A., Trujillo-Rivera, E.A., Arias-Trinidad, A. and Mendoza-López, M.R., 2016. Natural attenuation of weathered oil using aquatic plants in a farm in Southeast Mexico. International J. of Phytoremediation, 18, 877–884. doi.org/10.1080/15226514.2016.1156632

Sánchez-Arias, L.E., Avendaño, R.D., Álvarez-León, R., 2013. Evaluation of a recovery technique for mangrove soils affected by oil spills, using as indicator plantules of *Rhizophora mangle* L. (*Rhizophoraceae*). Pan-American Journal of Aquatic Sciences (2013), 8(2):79-88. https://www.researchgate.net/publication/279571495

Sarto, M.V.M., Borges, W.L.B., Bassegio, D., Pires, C.A.B., Rice, C.W. and Rosolem, C.A., 2020. Soil microbial community, enzyme activity, C and N stocks and soil aggregation as affected by land use and soil depth in a tropical climate region of Brazil. Arch Microbiol 202, 2809–2824. https://doi.org/10.1007/s00203-020-01996-8

SAS Institute Inc., 2004. Logistic regression examples using the SAS system version 9.4 first edition. McGraw Hill. Cary, N.C. 163 p

SEMARNAT, 2002. On the establishment of specifications for fertility, salinity and soil classification. Studies, sampling and analysis (official mexican standard that establishes the specifications of fertility, salinity and soil classification. Studies, sampling and analysis). NOM-021-RECNAT-2000. Official gazette of the Federation, Mexico.

SEMARNAT, 2012. Official mexican standard NOM-138-SEMARNAT/SSA1-2012 maximum permissible limits of hydrocarbons in soils and guidelines for sampling in the characterization and specifications for remediation.

SEMARNAT, 2012a. Mexican standard NMX-AA-134-SCFI-2006. Soils-heavy fraction hydrocarbons by extraction and gravimetry-test method. Official gazette of the Federation, Mexico

Semboung, L. F., Tarayre, C., Destain, J., Delvigne, F., Druart, P., Ongena, M. and Thonart, P., 2016. The effect of nutrients on the degradation of hydrocarbons in mangrove ecosystems by microorganisms. Int. J. Environ. Res. 10(4):583-592 ISSN: 1735-6865. https://www.researchgate.net/publication/311908267

Tahar, D., Tayeb, B., Chaabane, C., 2005. Chemical composition of essential oil of *Pinus halepensis* Miller growing in Algeria. Comptes rendus chimie, volume 8 (11-12) pp. 1939-1945.https://comptesrendus.academiesciences.fr/chimie/articles/10.1016/j.crci.2005.05.007/

Teutli-Hernández, C., Herrera-Silveira, J. A., and Cisneros-de la Cruz, D. (coord.). 2021. Manual for the ecological restoration of mangroves in the mesoamerican reef system and the wider Caribbean. Integrated ridge-to-reef management of the mesoamerican reef ecoregion project - MAR2R, UNEP-Cartagena convention, mesoamerican reef fund. Guatemala City, Guatemala. https://bvearmb.do/handle/123456789/1529

Thomas, N., Lucas, R., Bunting, P., Hardy, A., Rosenqvist, A., Simard, M., 2017. Distribution and drivers of global mangrove forest change, 1996–2010. PLOS ONE 12(6): e0179302. https://doi.org/10.1371/journal.pone.0179302

Truskewycz, A., Gundry, T.D., Khudur, L.S., Kolobaric, A., Taha, M., Aburto-Medina, A., Ball, A.S., Shahsavari, E., 2019. Petroleum hydrocarbon contamination in terrestrial ecosystems-fate and microbial responses. Molecules 19;24(18):3400.

Velázquez-Salazar, S. et al., 2021. Mangroves of Mexico update and analysis of 2020 data. National committee for the knowledge and use of biodiversity. Mexico CDMX. https://Manglares de México. Actualización y análisis de los datos 2020 (CONABIO) – Agua.org.mx. Accessed 11 May, 2024.

Verane, J., dos Santos, N.C.P., da Silva, V.L., de Almeida, M., de Oliveira, O.M.C. and Moreira, I.T.A., 2020. Phytoremediation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments using *Rhizophora mangle*. Marine Pollution Bulletin, volume 160, 111687, ISSN 0025-326X. https://doi.org/10.1016/j.marpolbul.2020.111687

Wadja, M.E. and Anthelme, G., 2021. Status and perspectives of mangrove management in Côte d'Ivoire. GSC Advanced Research and Reviews, 09(02), 045–050. DOI: https://doi.org/10.30574/gscarr.2021.9.2.0252

Zavala-Cruz, J., Castillo-Acosta, O., Ortiz-Ceballos, A., Palma-López, D.J., Juárez-López, J.F., Salgado-García, S., Rincón-Ramírez, J., Moreno-Cáliz, E., and Ramos-Muñoz, R., 2009. Capacity of urban land use in Tabasco, based on soil, current use and vegetation. http://www.libreroonline.com/mexico/libros/85587/zavala-cruz-joel-castillo-acosta-ofelia-ortiz-ceballos-angel-i-palma-lopez-david-j-juarez-lopez-/capacidad-de-uso-del-suelo-urbano-en-tabasco-con-base-en-suelo-uso-actual-y-vegetacion.html. Accessed 12 November 2023

Zeng, W.C., Zhang, Z., Gao, H., Jia, L.R. and He, Q., 2012. Chemical composition, antioxidant, and antimicrobial activities of essential oil from pine needle (*Cedrus deodara*). Journal of Food Science, 77: C824-C829. https://doi.org/10.1111/j.1750-3841.2012.02767.x