Chemical composition and adaptation of tropical grass *Leersia hexandra* Sw. exposed to crude oil soil

José Alberto Orocio-Carrillo [a*]

María del Carmen Rivera-Cruz [a]

Said Cadena-Villegas [a]

Consuelo del Carmen Bautista-Muñoz [a]

Antonio Juárez-Maldonado [b]

Karla Chávez-Álvarez [a]

[a] Colegio de Postgraduados. Campus Tabasco. Programa de Doctorado en Ciencias Agrícolas en el Trópico, Laboratorio de Microbiología Agrícola Ambiental. Periférico Carlos A. Molina, Km 3.5. 86500, H. Cárdenas, Tabasco, México.

[b] Universidad Autónoma Agraria Antonio Narro. Departamento de Botánica. Coahuila, México.

* Corresponding author: orocio.alberto@colpos.mx

Abstract:

The purpose was to evaluate the chemical composition and adaptation potential of L. *hexandra* under crude oil stress conditions, through the rhizobacterial population, crude protein accumulation, neutral detergent fiber, acid detergent fiber, and lignin in foliage of young plants emerging from the main tillers of the plant at different growth ages (d 180 and 360), as well as the production of young plants in the tillering stage, and the aerial and root dry matter. The results showed that crude oil concentrations in the soil significantly affected the population of *Azotobacter* spp. (0.361*); however, those of *Azospirillum* spp. and *Pseudomonas* spp. were inhibited, while both populations increased with time extension (0.778*, 0.767*). Likewise, the synthesis of crude protein (0.551**) and lignin in the foliage

(0.354*) and the production of young plants in the tillering stage (0.465**), as well as of root dry matter (0.362*) were increased, indicating a strategy of *L. hexandra* to survive and adapt to soil contamination by crude oil. Nevertheless, the chemical composition was affected by the age of the grass, in which the percentage of neutral detergent fiber (0.832**), acid detergent fiber (0.741**), and lignin (0.661**) increased, while that of crude protein decreased (-0.497**).

Keywords: Rhizobacteria, Crude Protein, Lignin, Neutral Detergent Fiber.

Introduction

Soils contaminated with crude oil have generated a toxic environment for plants and microbial activity in the rhizosphere of various plant species[1,2] due to petroleum's toxic, mutagenic, and carcinogenic nature[3]. Soil contamination with crude oil affects fertility in a negative way because crude oil decreases the availability of nutrients (nitrogen, phosphorus, and potassium), pH, and moisture content, and increases the electrical conductivity and organic carbon content of the soil[4] directly affecting plant growth and development[5]. In addition, the oil also forms a hydrophobic surface around the roots, which limits the absorption of water and nutrients from the soil[6]. In oiled soil, *Leersia hexandra* grows and produces forage[7]; it is a perennial grass that inhabits humid areas in tropical and subtropical regions; it propagates vegetatively by rhizomes, invading wetlands by the abundant accumulation of its foliage and roots on the soil and water[8] and is highly consumed by cattle in both dry and rainy seasons[9].

The rhizosphere is the soil-root interface, the volume of soil influenced by root activity[10]. Tropical grasses are characterized by an extended rhizosphere system due to the abundant fibrous root system that characterizes them, and have demonstrated adaptation to the stress conditions established in soils contaminated with crude oil[11]. Tropical grasses that have naturally evolved under stress conditions due to the accumulation of biogenic and petroleum carbon in the soil are able to mitigate stress by changing the chemical conditions in the rhizosphere and the proliferation of specific heterotrophic microorganisms[12,13]. The rhizobacteria are free-living heterotrophs associated with the plant root[14]; they stimulate

plant growth, provide plant tolerance to petroleum diesel stress[15], and are involved in the rhizodegradation processes of alkanes, cycloalkanes, and polycyclic aromatic hydrocarbons[16]. *Azotobacter*, *Azospirillum*, and *Pseudomonas* bacterial genera tolerate crude oil-induced stress[17,18]. Plant growth-promoting rhizobacteria that colonize the rhizosphere mineralize intermediate metabolites from the decomposition of organic carbon in petroleum[19], and the exoenzymes released by them reduce and oxidize nitrogen, phosphorus, and sulfate, but also fix nitrogen biologically[20]. The adaptation of grasses to crude oil soil suggests that it has a rhizosphere with roots that assimilate NO_3, H_2PO_4, and SO_4, and are used in an essential way in the primary metabolism inside the plant[21]. Grasses adapted to crude oil exposure are likely to respond in a similar manner to other plant species such as *Banksia seminuda* Rie., *Hakea prostrata* R. Br.[22], and *Secale cereal* L.[23] where abiotic stress modifies the development and expression of genes involved in cell synthesis in the roots and foliage[24]. The adaptation of plastids to stress by abiotic factors is based on the positive response of growth, production, and synthesis of secondary metabolites. In this regard, Orocio-Carrillo *et al*[13] report that the root and leaf protein content of *L. hexandra* has a hormetic response to doses of total petroleum hydrocarbons in the soil. Correa and Maranho[25] indicated that stem length, root biomass, and stomatal density increase in *Echinochloa polystachya* (Kunth) Hitchc. exposed to oil-containing soil; Habermann *et al*[26] indicate that water deficit stress and soil heating induced an increase in fiber and lignin content and reduced leaf protein in *Panicum maximum* Jacq; other authors[27,28] report that high temperatures lead to high detergent fiber and lignin content in the cell wall, and decreased protein synthesis in forage grasses. The objective of this study was to determine the rhizobacterial population, crude protein accumulation, neutral detergent fiber, acid detergent fiber, and lignin in the foliage, as well as the production of young *L. hexandra* plants in the tillering stage and in their aerial and root dry matter, to obtain a grass adapted to soils contaminated with crude oil for the Mexican humid tropics.

Material and methods

Soil and rhizome collection

Uncontaminated soil (Gleysol) was collected from the surface layer (0-30 cm) located in *Ejido* Blasillo 4th Section, Huimanguillo, Tabasco, Mexico (18° 05' 08.4" N and 93° 56' 50" W). The soil was dried under shade, ground, and sieved (5 mm mesh). The physical and chemical characteristics of the soil are shown in Table 1. *L. hexandra* rhizomes were collected from a wetland affected by a chronic oil spill, located two kilometers southwest of the "La

Venta" Gas Processing Complex. The cultivation of *L. hexandra* seedlings was similar to the procedure used by Orocio-Carrillo *et al*[13].

Table 1: Physical and chemical characteristics of soil and oil

Soil characteristics										
Texture	Sand	Silt	Clay	pH	OM	TN	SO_4^{2-}	PO_4-	EC	CEC
Loamy clayey sandy	45.2%	20.7%	34.1%	5.5	17.2%	0.66%	45 mg kg^{-1}	85 mg kg^{-1}	0.9 dS m^{-1}	9.6 cmol kg^{-1}

Characteristics of petroleum[a]				
API Gravity	Sulphur	Factions		
32°	1.8%	Saturated 61.2 %	Aromatic 24.8 %	Asphaltenes + Resins 14 %

OM= organic matter; TN= total nitrogen; EC= electrical conductivity; CEC= cation exchange capacity.
[a]Hydrocarbon fractions using the Soxhlet and gravimetric methods.

Soil contamination and experimental design

32° API crude oil (CO) was obtained from the Ogarrio Field, Battery 2, in Huimanguillo, Tabasco, Mexico. The experiment was carried out with a completely randomized design and a 4x2 factorial arrangement: four concentrations of CO [0 (control), 30, 60, and 90 g kg^{-1} DW (dry weight)] and two exposure times of *L. hexandra* to CO= (d 180 and 360). A total of eight treatments with four replicates maintained at random locations in a microtunnel with an average temperature of 29 ± 6 °C and humidity at field capacity of 32 ± 5 %. The experimental unit was a plastic container with 4 kg of dry soil and one *L. hexandra* plant.

Rhizobacterial population

Azospirillum bacteria were grown on Congo red agar[29], *Azotobacter* bacteria on Asby agar(29), and *Pseudomonas* bacteria on cetrimide + glycerol agar[30]. Cultures were incubated at 28 °C for 72 h, and counts were expressed as colony forming units (CFU) per gram of soil.

Chemical composition of *L. hexandra*

Destructive sampling of plant tissue (leaves and stems) from young plants emerging from the main plant of *L. hexandra* that were 180 and 360 days old was performed, dried in a forced air oven at 60 °C for 72 h and ground for crude protein, neutral detergent fiber, acid detergent fiber, and lignin analysis[31,32].

Production analysis

The evaluation of young plants in the tillering stage, aerial dry matter, and root dry matter was similar to the procedure used by Orocio-Carrillo *et al*[33].

Statistical analyses

The data collected for all variables were subjected to an analysis of variance and a multiple comparison test of means with Tukey's method ($P \leq 0.05$), as well as to Pearson's bivariate correlation, using the SAS v.9.4 statistical software[34].

Results

Rhizobacterial population

Table 2 shows the changes in the mean values of the three groups of rhizobacteria due to the effect of crude oil on the soil and to the exposure time. The highest density of *Azotobacter* [438 and 132 x 103 CFU g^{-1} dry rhizosphere (d.r.)] was found in soil with 90 g kg^{-1} of crude oil, where it was 106.6 % and 40.4 % higher than the control at d 180 and 360 respectively. However, the densities of *Azospirillum* and *Pseudomonas* decreased. In general, the effect of crude oil contamination increases in the population of *Azotobacter* (81.7 %) and decreases in those of *Azospirillum* and *Pseudomonas* by 36 and 47.7 %, respectively. However, the effect of the time of evaluation induced a positive response in the density of *Azospirillum* and *Pseudomonas*, being 1.9 and 23.9 times higher at d 360 than at d 180.

Table 2: Changes in *Azospirillum*, *Azotobacter*, and *Pseudomonas* in the rhizosphere of *L. hexandra* exposed to crude oil at 180 and 360 days

Time/Crude oil (g kg⁻¹)	*Azospirillum*	%	*Azotobacter*	%	*Pseudomonas*	%
	10^3 UFC g⁻¹ r.s.				10^1 UFC g⁻¹ r.s.	
180 days						
0	84 c		212 c		95 c	
30	67 d	−20.2	372 b	+75.5	58 c	−38.9
60	53 e	−36.9	482 a	+127.3	34 c	−64.2
90	21 f	−75.0	438 a	+106.6	74 c	−22.1
360 days						
0	144 a		94 e		2,427 a	
30	101 b	−29.9	85 e	−9.6	2,335 a	−3.8
60	102 b	−29.2	159 d	+69.1	572 bc	−76.4
90	95b c	−34.0	132 de	+40.4	889 b	−63.4
Contamination						
Without	114*		153		1,261*	
With	73	−36	278*	+81.7	660	−47.7
Time (days)						
180	56		376*		65	
360	110*		117		1,556*	

The symbol % (+) represents an increase, and % (–), a decrease of *Azospirillum*, *Azotobacter*, and *Pseudomonas* at 180 and 360 d with respect to the values of the control treatment (0 g kg⁻¹ crude oil). [abcde] Different lowercase letters within a column indicate statistically different values ($P \leq 0.05$, n=4). * Statistically higher.

Chemical composition of *L. hexandra*

Figure 1 shows the tendency in crude protein content in the aerial dry matter of young *L. hexandra* plants by effect of crude oil dosage and exposure time. The highest crude protein content at d 180 (9.1 %) and 360 (8.4 %) was recorded at the 90 g kg⁻¹ crude oil concentration, being 1 and 1.5 % higher than that of the control (Figure 1a). On the other hand, the

evaluation time decreased from 8.4 to 7.5 % as the age of the plant increased from 180 to 360 d (Figure 1b).

Figure 1: Effect of the crude oil in the soil (a) and exposure time (b) on the crude protein percentage in *L. hexandra*
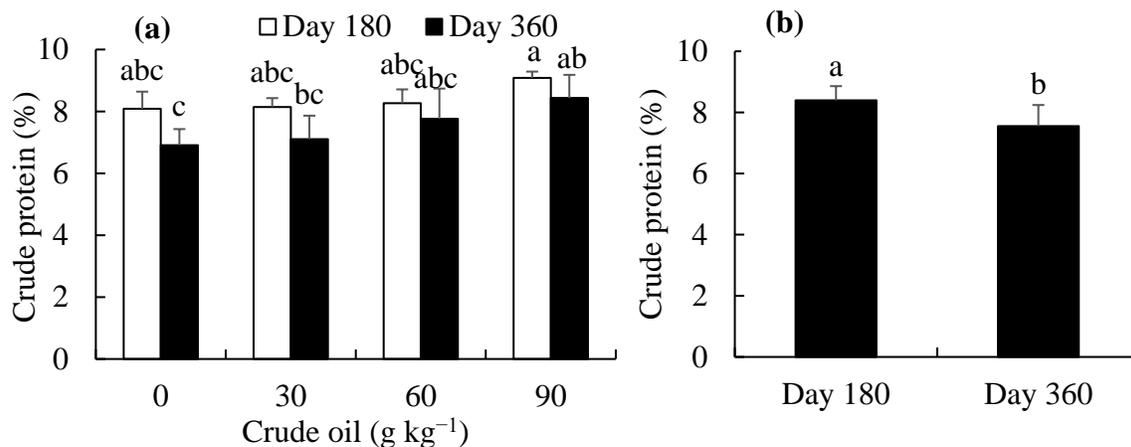
[abcd] Different letters indicate different values ($P \leq 0.05$, n=4).

Figure 2 shows the performance in neutral detergent fiber. Significant differences were observed according to the oil dose and the evaluation time ($P \leq 0.05$). The dose of 90 g kg^{-1} of crude oil induced the highest neutral detergent fiber content at d 180 (75.3 %) and 360 (79.3 %), reaching an increase of 1.3 and 2.5 % over that of the control (Figure 2a). As for the evaluation time, it increased from 73.9 to 77.9 % as plant maturity increased from 180 to 360 d (Figure 2b).

Figure 2: Effect of crude oil in the soil (a) and the exposure time on the neutral detergent fiber in *L. hexandra*
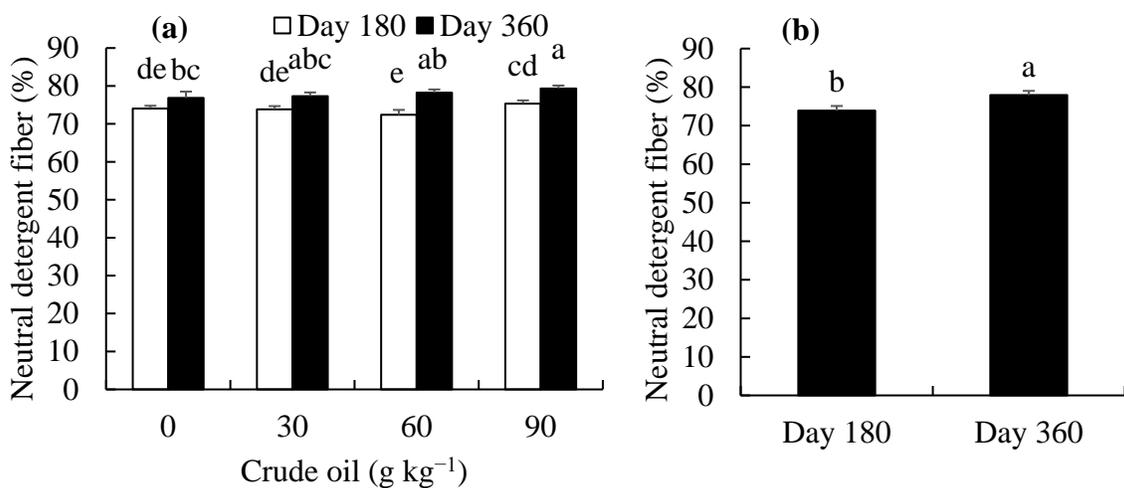
[abcd] Different letters indicate different values ($P \leq 0.05$, n=4).

Figure 3 shows the behavior in the content of acid detergent fiber. At day 180, the dose of crude oil was observed to stimulate the acid detergent fiber, which was higher (41.3 %) in soil with 90 g kg^{-1} of crude oil, increasing 6.4 % over that of the control (Figure 3a). At day 360, crude oil concentrations showed a statistically similar response to the control. As for the evaluation time, the acid detergent fiber was stimulated to increase from 36.8 to 43.6 % at day 180 and 360, respectively (Figure 3b).

Figure 3: Effect of the crude oil on the soil (a) and time of exposure (b) on the acid detergent fiber in *L. hexandra*

abcd Different letters indicate different values (*P*≤0.05, n=4).
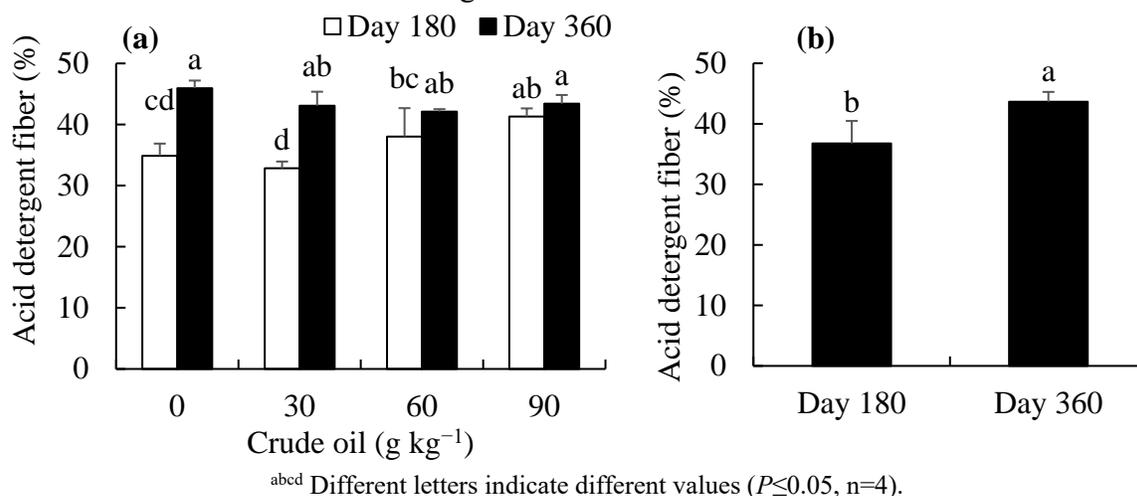
The effect of crude oil on the lignin content in *L. hexandra* harvested at different ages showed significant differences (*P*≤0.05) (Figure 4). At d 180, it is observed that high concentrations of oil stimulate lignin, being higher (4.9 %) in soil with 90 g kg^{-1} of crude oil, increasing 1 % with respect to the control (Figure 4a). On the other hand, the effect of evaluation time induced a positive response, reaching an increase of 4.3 to 5 % as plant age advanced (Figure 4b).

Figure 4: Effect of crude oil on the soil (a) and exposure time (b) on the percentage of lignin in *L. hexandra*

abcd Different letters indicate different values ($P \leq 0.05$, n=4).
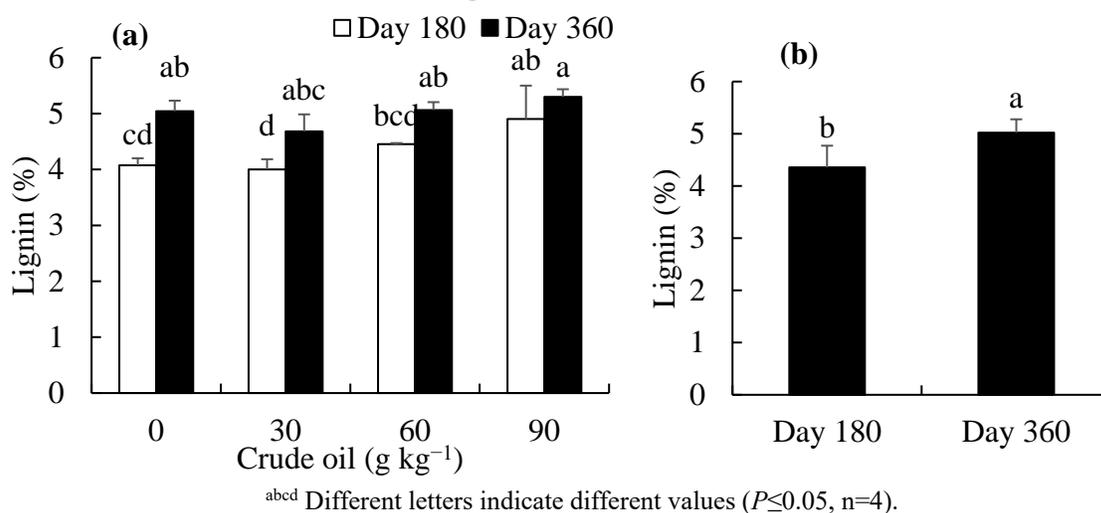
L. hexandra production

The effects of crude oil doses and evaluation time on the means of young *L. hexandra* plants in the tillering stage and their aerial dry matter and root dry matter exhibited significant differences ($P<0.05$) (Table 3). Exposure to 90 g kg^{-1} of crude oil promoted an increase of up to 300 and 89.3 % of young plants in the tillering stage compared to the control soil at d 180 and 360 respectively. The results for root dry matter increased up to 203.7 and 169.7 % at d 180 and 360, respectively, in the most contaminated soil, with respect to the control soil. However, the aerial dry matter production of the grass decreased up to 25.9 and 18.9 % at day 180 and 360 with the highest dose. In general, the effect of crude oil contamination on young plants in the tillering stage increases by 78.6 % and radical dry matter 135.1 %. However, it decreased 13.4 % in the aerial dry matter, although this reduction was statistically equal to the control soil. The effect of the evaluation time induced a positive response in young *L. hexandra* plants in the tillering stage and the aerial and root dry matter, which were 210.8, 378.7, and 545 % higher at d 360 than at d 180.

Table 3: Changes in young plants in the tillering stage, the aerial dry matter and the root dry matter of *L. hexandra* exposed to crude oil at 180 and 360 days

Time/ Crude oil (g kg⁻¹)	Young plants in tillering stage	%	Aerial dry matter (g)	%	Root dry matter (g)	%
180 days						
0	27±2.4 f		43.5±2.3 d		2.7±0.2 d	
30	52±5.1 e	+92.6	42.2±2.5 d	−2.9	6.2±0.3 d	+129.6
60	71±12.1 e	+163	36.2±4.2 d	−16.8	7.0±1.4 d	+159.3
90	108±4.2 d	+300	32.2±5.3 d	−25.9	8.2±0.3 d	+203.7
360 days						
0	140±4.3 c		204.2±8.1 a		19.5±2.1 c	
30	191±6.9 b	+36.4	188.6±9.3 b	−7.6	39.2±3.8 b	+101
60	210±8.7 b	+50	178.7±4.9 bc	−12.5	43.5±5.3 b	+123.1
90	265±17.8 a	+89.3	165.6±7.7c	−18.9	52.6±2.8 a	+169.7
Contamination						
Without	84		123.9*		11.1	
With	150*	+78.6	107.3*	−13.4	26.1*	+135.1
Time (days)						
180	65		38.5		6.0	
360	202*	+210.8	184.3*	+378.7	38.7*	+545

The symbol % (+) represents an increase, and % (-) a decrease, of the aerial dry matter and root dry matter of young plants at d 180 and 360, compared to the values of the control treatment (0 g kg⁻¹ of crude oil).

[abcde] Different lowercase letters within each column represent different values ($P \leq 0.05$, n=4). *Statistically higher.

Correlation of variables are presented in Table 4.

Discussion

Rhizobacterial population

Reports indicate that the rhizosphere of grasses used for the removal of total petroleum hydrocarbons from soils contaminated with crude oil host intense microbial activity, including that of plant growth-promoting rhizobacteria[35,36]. The significant increase in the *Azotobacter* population (0.361*) (Table 2 and 4) in the rhizosphere of *L. hexandra* is similar

to that reported in other studies evaluating the same grass exposed to 60-180 g kg^{-1} of total petroleum hydrocarbons[20] and *Echinochloa polystachya* K. exposed to 65.89 g kg^{-1} of total petroleum hydrocarbons[37]. The increase in the *Azotobacter* population could be due to the adaptation of the bacterium through the secretion of extracellular enzymes essential for the initial degradation of high molecular weight substrates[38] such as petroleum hydrocarbons. Similarly, it may be an adaptation to a reduction in the availability of essential nutrients such as nitrogen due to the properties of crude oil, which tends to agglomerate in the soil[4;5]. In addition, it has been shown that under stress conditions plants increase root exudation[39], which become a source of nutrients and stimulating substances for the growth of microorganisms[40]. However, not all microorganisms can adapt quickly, probably because of the toxic hydrocarbons in crude oil, which make it difficult for some microbial species to grow and survive[41]. In addition, the presence of oil can modify the physicochemical properties of the soil[4], generating unfavorable conditions for microorganisms[42]. In contrast, the current study revealed that the population of *Azospirillum* and *Pseudomonas* decreased with increased crude oil, demonstrating the negative effects of this pollutant; at the same time, with the extension of time, the stimulation of both populations was promoted (0.778**, 0.767**), indicating that the bacteria need some time to acclimatize and achieve significant growth. Similar results were reported in other investigations[1,7], where they found a significant increase of rhizobacteria over time in the rhizosphere of *L. hexandra* and *Urochloa brizantha* Hochst exposed to oil.

Chemical composition of *L. hexandra*

The present study showed increases in crude protein content in shoots of *L. hexandra* planted in soil with crude oil (0.551**) relative to the control. At d 180 and 360 at doses of 60 and 90 g kg^{-1} of crude oil, the tendency is for the crude protein to increase with respect to the control, while at doses of 30 g kg^{-1} there was no statistical difference (Figure 1a). A similar effect was reported by Orocio-Carrillo *et al*[13], who found an increase in crude protein in *L. hexandra* exposed to 102 g kg^{-1} of crude oil. Likewise, the protein content in *Simmodsia chinensis* L. and *Vigna unguiculata* L. respectively has reportedly[43,44] increased due to the effect of crude oil in soil. Roa *et al*[45] report an increase in protein concentration in *Triticum aestivum* L. due to the effect of sulfur fertilization. Therefore, the increase in protein in this study could be due to the nitrogen and sulfur content present in the crude oil[46]. On the other hand, crude protein is diluted as the age of the grass increases (−0.497**). It has been mentioned[47] that the increase in the age of the grass results in a decrease in protein, as dry matter production increases. In this regard, this study found a negative and highly significant relationship between crude protein and aerial dry matter (−0.564**). As the maturity stage

of grasses increases, so does the content of structural carbohydrates and lignin, while the protein content decreases[48].

This study shows that the neutral detergent fiber at d 180 did not change in contaminated soils with respect to the control; however, at d 360, the tendency was to increase very slightly with high doses of crude oil (60 and 90 g kg^{-1}), but at 30 g kg^{-1} there was no statistical difference compared to the control. On the other hand, neutral detergent fiber increased due to the effect of the evaluation time (0.832**). Similar tendencies were reported by other researchers[49,50], who evaluated the percentages of neutral detergent fiber of fountaingrass at different harvesting ages and observed an increase in its concentration of 5.5 and 17.9 % between day 30 and 167 d, respectively. It has also been mentioned[51] that, as forage maturity increases, the concentrations of neutral detergent fiber in stems and leaves augment, reducing the voluntary consumption of forages.

The concentration of acid detergent fiber in this study shows that it increased at high doses of oil (60 and 90 g kg^{-1}) with respect to the control at d 180, but at doses of 30 g kg^{-1} the response was not statistically different from that of the control. At d 360, the tendency was to decrease at doses of 60 and 90 g kg^{-1}, while at doses of 30 g kg^{-1} there were no statistical differences in relation to the control. On the other hand, the concentration of acid detergent fiber augmented as the age of the grass increased (0.741**). Similar values were reported by Schnellmann *et al*[52] when evaluating the nutritional quality of *Megathyrsus maximus* Jacq., as they recorded values of 29.3 % at d 90 and 34.4 % at d 180. Similarly, Álvarez-Vázquez *et al*[50] found an increase of 40.36 % at d 33 and 58.5 % at d 180 when evaluating the chemical composition of *Cenchrus* sp. grass. Acid detergent fiber is reported to be an important component that regulates forage quality and is positively related to the crop's age or stage of development, with forage quality declining as fiber becomes a predominant component[53].

In the present study, one explanation for the increase in high doses of crude oil may be the mechanism of osmotic adjustment that favors the accumulation of compatible solutes, which are organic compounds that do not interfere with cell metabolism, even at high concentrations, and can act as antioxidants to minimize the impact of abiotic stress on the plant[24]. Several studies have shown that lignin increases in response to various environmental stresses[26,27], playing a role in the adaptation of plants to their environment[54]. On the other hand, the lignin content is stimulated (0.661**) as the age of maturity of the plant increases. Rosales and Pinzón[48] mentioned that, as the grass maturity stage increases, the proportion of cell wall components, including lignin, increases, reducing the digestibility of the grass.

L. hexandra production

In pastures, the addition of crude oil to the soil has been reported to induce a significant reduction of plant dry matter[55]. It should be noted that, at d 180, aerial dry matter production was reduced only by 2.9 % at a concentration of 30 g kg^{-1} of crude oil, compared to the control, demonstrating the high tolerance of the grass in these conditions; however, as the dose of crude oil in the soil increases (60 and 90 g kg^{-1}), the percentage reduction increases both at d 180 and at d 360 with respect to the control. The negative impact of crude oil on the aerial dry matter of *L. hexandra* has also been reported[13,56]. Crude oil leads to negative changes in soil properties such as reduced moisture absorption and retention capacity, water repellency and insufficient soil aeration[57,58], which consequently makes plant growth even more difficult. On the other hand, the production of young plants in the tillering stage and root dry matter were stimulated (0.465**, 0.362*). This behavior was also mentioned in other researches[13,56]. The increase in these variables may indicate a strategy of *L. hexandra* to survive and adapt to soil contamination by crude oil. The increase of young plants in the tillering stage and root dry matter in oiled soils could be explained by the increase in soil organic carbon content due to the degradation of crude oil[18]. Likewise, the increase in roots could be due to a response of reinforcing the root tissues in order to limit soil nutrient deficiency stress induced by crude oil[5]. Studies have reported increased root biomass in soils treated with petroleum hydrocarbons[22].

Conclusions and implications

The effect of high doses of crude oil in the soil induces in the rhizosphere of *L. hexandra* high populations of bacteria of the *Azotobacter* group; however, it caused the inhibition of *Azospirillum* and *Pseudomonas*. On the other hand, crude oil is positively correlated with crude protein and lignin synthesis, playing a role in grass adaptation to its environment. Likewise, high doses of crude oil induced a positive response in the production of young plants in the tillering stage and root dry matter, which indicates a strategy developed by the grass to survive and adapt to oil contamination. However, the chemical composition of the grass was affected by the age of harvest, in which the percentage of neutral detergent fiber, acid detergent fiber, and lignin increased, while the concentration of crude protein decreased. Therefore, even if the grass is able to adapt to the stress induced by crude oil, its protein content and, therefore, its nutritional value are affected, which can be detrimental to farmers and cause long-term deterioration of the affected property.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

Literature cited:

1. Alarcón A, García-Díaz M, Hernández-Cuevas LV, Esquivel-Cote R, Ferrera-Cerrato R, Almaraz-Suarez JJ, *et al*. Impact of crude oil on functional groups of culturable bacteria and colonization of symbiotic microorganisms in the *Clitoria-Brachiaria* rhizosphere grown in mesocosms. Acta Biol Colomb 2019;24(2):343-353.

2. Zawierucha I, Malina G, Herman B, Rychter P, Biczak R, Pawlowska B, *et al*. Ecotoxicity and bioremediation potential assessment of soil from oil refinery station area. J Environ Health Sci Eng 2022;20:337-346.

3. Sattar S, Hussain R, Shah SM, Bibi S, Ahmad SR, Shahzad A, *et al*. Composition, impacts, and removal of liquid petroleum waste through bioremediation as an alternative clean-up technology: A review. Heliyon 2022;8:e11101.

4. Devatha CO, Vishnu VA, Chandra RJP. Investigation of physical and chemical characteristics on soil due to crude oil contamination and its remediation. Appl Water Sci 2019;9:89.

5. Correa HS, Blum CT, Galvão F, Maranho LT. Effects of oil contamination on plant growth and development: a review. Environ Sci Pollut Res Int 2022;29:43501-43515.

6. Adams RH, Zavala-Cruz J, Morales-García F. Concentración residual de hidrocarburos en suelo del trópico. II: Afectación a la fertilidad y su recuperación. Interciencia 2008;33(7):483-489.

7. Rivera-Cruz MC, Trujillo-Narcía A, Trujillo-Rivera EA, Arias-Trinidad A, Remedios Mendoza-López MR. Natural attenuation of weathered oil using aquatic plants in a farm in Southeast Mexico. Int J Phytoremediation 2016;18(9):877-884.

8. Vegetti AC. Caracterización de los sistemas de ramificación en especies de *Oryzeae* (Poaceae). Candollea 2002;57(2):251-260.

9. Aparicio R, González-Ronquillo M, Torres R, Astudillo L, Córdova L, Carrasquel J. Degradabilidad de los pastos lambedora (*Leersia hexandra*) y paja de agua (*Hymenachne amplexicaulis*) en cuatro épocas del año de una sabana inundable del estado Apure, Venezuela. Zootec Tropic 2007;25(3):225-228.

10. Neumann G, Römheld V. Rhizosphere chemistry in relation to plant nutrition. In: Marschner P editor. Marschner's mineral nutrition of higher plants. 3rd ed. San Diego: Academic Press; 2012:347-368.

11. Panchenko L, Muratova A, Dubrovskaya E, Golubev S, Turkovskaya O. Dynamics of natural revegetation of hydrocarbon-contaminated soil and remediation potential of indigenous plant species in the steppe zone of the southern Volga Uplands. Environ Sci Pollut Res 2018;25:3260-3274.

12. Correa-García S, Pande P, Séguin A, St-Arnaud M, Yergeau E. Rhizoremediation of petroleum hydrocarbons: a model system for plant microbiome manipulation. Microb Biotechnol 2018;11:819-832.

13. Orocio-Carrillo JA, Rivera-Cruz MC, Aranda-Ibañez EM, Trujillo-Narcía A, Hernández-Galvez G, Mendoza-López MR. Hormesis under oil-induced stress in *Leersia hexandra* Sw. used as phytoremediator in clay soils of the Mexican humid tropic. Ecotoxicology 2019;28:1063-1074.

14. Minuț M, Diaconu M, Roșca M, Cozma P, Bulgariu L, Gavrilescu M. Screening of *Azotobacter*, *Bacillus* and *Pseudomonas* species as plant growth-promoting bacteria. Processes 2022;11:80.

15. Graj W, Lisiecki P, Szulc A, Chrzanowski Ł, Wojtera-Kwiczor J. Bioaugmentation with petroleum-degrading consortia has a selective growth-promoting impact on crop plants germinated in diesel oil-contaminated soil. Water Air Soil Pollut 2013;224:1676.

16. Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ. Rhizoremediation: A beneficial blant-microbe interaction. Mol Plant Microbe Interact 2004;17(1):6-15.

17. Arias-Trinidad A, Rivera-Cruz MC, Roldán-Garrigós A, Aceves-Navarro LA, Quintero-Lizaola R, Hernández-Guzmán J. Uso de *Leersia hexandra* (Poaceae) en la fitorremediación de suelos contaminados con petróleo fresco e intemperizado. Rev Biol Trop 2017;65(1):21-30.

18. Chávez-Álvarez K, Rivera-Cruz MC, Aceves-Navarro LA, Trujillo-Narcía A, García-de la Cruz R, Vega-López A. Physiological and microbiological hormesis in sedge *Eleocharis palustris* induced by crude oil in phytoremediation of flooded clay soil. Ecotoxicology 2022;31(8):1241-1253.

19. Khan S, Afzal M, Iqbal S, Khan QM. Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. In: Chemosphere. Elsevier Ltd. 2013;1317-1332.

20. Silva PVC, Plucani AF, Ané JM, Stacey G. Diazotrophic bacteria and their mechanisms to interact and benefit cereals. Curr Rev 2021;34(5):491-498.

21. Kochhar SL, Kaur GS. Plant physiology: Theory and applications. 2nd ed. Cambridge University Press; 2020.

22. Hoang SA, Lamb D, Seshadri B, Sarkar B, Cheng Y, Wang L, *et al*. Petroleum hydrocarbon rhizoremediation and soil microbial activity improvement via cluster root formation by wild proteaceae plant species. Chemosphere 2021;275:130135.

23. Skrypnik L, Maslennikov P, Novikova A, Kozhikin M. Effect of crude oil on growth, oxidative stress and response of antioxidative system of two rye (*Secale cereale* L.) varieties. Plants 2021;10(1):157.

24. Shinozaki K, Uemura M, Bailey-Serres J, Bray AE, Weretilnyk E. Responses to abiotic stress. In: Buchanan B, *et al*, editors. Biochemistry and molecular biology of plants. 2nd ed. Wiley and Blackwell, Chichester; 2015:1051-1100.

25. Correa HS, Maranho LT. The potential association of *Echinochloa polystachya* (Kunth) Hitchc. with bacterial consortium for petroleum degradation in contaminated soil. Appl Sci 2021;3:80.

26. Habermann E, Dias OEA, Contin DR, Delvecchio G, Viciedo DO, de Moraes MA, *et al*. Warming and water deficit impact leaf photosynthesis and decrease forage quality and digestibility of a C4 tropical grass. Physiol Plant 2019;165(2):383-402.

27. Lee MA, Davis AP, Chagunda MGG, Manning P. Forage quality declines with rising temperatures, with implications for livestock production and methane emissions. Biogeosciences 2017;14:1403-1417.

28. Dumont B, Andueza D, Niderkorn V, Lüscher A, Porqueddu C, Picon-Cochard C. A meta-analysis of climate change effects on forage quality in grasslands: specificities of mountain and Mediterranean areas. Grass Forage Sci 2015;70:239-254.

29. Döbereiner J, Marriel IE, Nery M. Ecological distribution of *Spirillum lipoferum* Beijerinck. Can J Microbiol 1966;22:1464-1473.

30. Garrity GM, Bell JA, Lilburn T. Order IX. Pseudomonadales Orla-Jensen 1921, 270AL. In: Brenner DJ, *et al*. editors. 2nd ed. Bergey's Manual® of Systematic Bacteriology. Boston, Springer; 2005:323-442.

31. AOAC. Official methods of analysis. 15th ed. Arlington, VA, USA: Association of Official Analytical Chemists. 1990.

32. Van Soest JP. Nitrogen metabolism. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press: Ithaca, NY: Comstock Publishing Associates; 1994.

33. Orocio-Carrillo JA, Rivera-Cruz MC, Juárez-Maldonado A, Bautista-Muñoz CC, Trujillo-Narcía A, González-García Y, *et al*. Crude oil induces plant growth and antioxidant production in *Leersia hexandra* Sw. Plant Soil Environ 2024;70(2):72-83.

34. SAS. Institute. SAS. Statistic User's guides Statics. SAS Inst. Inc 2016.

35. Rodríguez-Rodríguez N, Rivera-Cruz MC, Trujillo-Narcía A, Almaráz-Suárez JJ, Salgado-García S. Spatial distribution of oil and biostimulation through the rhizosphere of *Leersia hexandra* in degraded soil. Water Air Soil Pollut 2016;227(9):319.

36. Uribe M, Peñuela GA, Pino NJ. *Megathyrsus maximus* and *Brachiaria decumbens* improve soil characteristics and select promising rhizobacteria during rhizoremediation of petroleum hydrocarbons. Rhizosphere 2022;22:100517.

37. Rivera-Cruz MC. Flora y microflora rizosférica del pantano: indicadoras de perturbación causada por petróleo e inundación. Agroregión 2011;5:12-15.

38. Badalucco L, Kuikman PJ. Mineralization and Immobilization in the Rhizosphere. In: Pinton R, *et al*, editors. The rhizosphere. Biochemistry and organic substances at the soil-plant interface. United States of America: Marcel Dekker AG; 2001:159-196.

39. Xie X, Weiss DJ, Weng B, Liu J, Lu H, Yan C. The short-term effect of cadmium on low molecular weight organic acid and amino acid exudation from mangrove (*Kandelia obovata* (S., L.) Yong) roots. Environ Sci Pollut Res 2013;20:997-1008.

40. Zhao M, Zhao J, Yuan J, Hale L, Wen T, Huang Q, *et al*. Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth. Plant Cell Environ 2021;44(2):613-628.

41. Huang L, Ye J, Jiang K, Wang Y, Li Y. Oil contamination drives the transformation of soil microbial communities: Co-occurrence pattern, metabolic enzymes and culturable hydrocarbon-degrading bacteria. Ecotoxicol Environ Saf 2021;225:112740.

42. Bidja AMT, Chen G, Chen Z, Zheng X, Li S, Li T, *et al*. Microbial diversity changes and enrichment of potential petroleum hydrocarbon degraders in crude oil-, diesel-, and gasoline-contaminated soil. Biotech 2020;10(2):42.

43. Al-Hawas GHS, Shukry WM, Azzoz MM, Al-Moaik RMS. The effect of sublethal concentrations of crude oil on the metabolism of Jojoba (*Simmodsia chinensis*) seedlings. Int Res J Plant Sci 2012;3(4):54-62.

44. Achuba FI, Ja-anni MO. Effect of abattoir waste water on metabolic and antioxidant profiles of cowpea seedlings grown in crude oil contaminated soil. Int J Recycl Waste Agric 2018;7:59-66.

45. Roa GA, Quintana-Obregón EA, González-Renteria M, Diaz DAR. Increasing wheat protein and yield through sulfur fertilization and its relationship with nitrogen. Nitrogen 2024;5(3):553-571.

46. Tissot BP, Welte DH. Petroleum formation and occurrence: composition of crude oils. 2nd ed. Berlin Heidelberg, New York: Springer-Verlag; 1984.

47. Juárez J, Bolaños ED, Vargas LM, Medina S, Martínez-Hernández PA. Curvas de dilución de la proteína en genotipos del pasto *Brachiaria humidicola* (Rendle) Schweick. Rev Cub Cien Agric 2011;45(3):321-331.

48. Rosales RB, Pinzón SS. Limitaciones físicas y químicas de la digestibilidad de pastos tropicales y estrategias para aumentarla. Rev Corp 2005;6(1):69-82.

49. Palacios-Díaz MP, Mendoza-Grimón V, Fernández-Vera JR, Hernández-Moreno JM. Effects of defoliation and nitrogen uptake on forage nutritive values of *Pennisetum* sp. J Anim Plant Sci 2013;23(2):566-574.

50. Álvarez-Vázquez P, Mendoza-Pedroza SI, Cadena-Villegas S, Calzada-Marín JM, Ortega-Jiménez E, Vaquera-Huerta H, *et al*. Cambios en el rendimiento y composición química del pasto Maralfalfa (*Cenchrus* sp) a diferente edad. Rev Fitotec Mex 2021;44(4):729-736.

51. Grev AM, Wells MS, Catalano DN, Martinson KL, Jungers JM, Sheaffer CC. Stem and leaf forage nutritive value and morphology of reduced lignin alfalfa. Agron J 2020;112:406-417.

52. Schnellmann LP, Verdoljak JJO, Bernardis A, Martínez-González JC, Castillo-Rodríguez SP, Limas-Martínez AG. Cutting frequency and height on the quality of *Megathyrsus maximus* (cv. Gatton panic). Cien Tecnol Agropecu 2020;21(3):e1402.

53. Horrocks RD, Vallentine JF. Field-Harvesting hay. Harvest Forag 1999;245-277.

54. Han X, Zhao Y, Chen Y, Xu J, Jiang C, Wang X, *et al*. Lignin biosynthesis and accumulation in response to abiotic stresses in woody plants. J Forestry Res 2022;2:9.

55. Wei Y, Wang Y, Duan M, Han J, Li G. Growth tolerance and remediation potential of six plants in oil-polluted soil. J Soil Sediment 2019;19(11):3773-3785.

56. González-Moscoso M, Rivera-Cruz MC, Delgadillo-Martínez J, Lagunes-Espinosa LC. Growth analysis and plant production of *Leersia hexandra* Swartz in tropic wet Mexican in function on petroleum and surfactant. Polibotánica 2017;43:177-196.

57. Marín-García DC, Adams RH, Hernández-Barajas R. Effect of crude petroleum on water repellency in a clayey alluvial soil. Int J Environ Sci Technol 2016;13:55-64.

58. Stepanova AY, Gladkov EA, Osipova ES, Gladkova OV, Tereshonok DV. Bioremediation of soil from petroleum contamination. Processes 2022;10(6):1224.

Table 4: Correlation of variables

Parameter	Time	PJM	ADM	RDM	CP	NDF	ADF	Lignin	AZP	AZT	PSE
CO	NS	0.465**	NS	0.362*	0.551**	NS	NS	0.354*	−0.558**	0.361*	−0.372*
Time		0.872**	0.987**	0.876**	−0.497**	0.832**	0.741**	0.661**	0.778**	−0.857**	0.767**
PJM			0.798**	0.966**	NS	0.860**	0.690**	0.739**	0.416*	−0.614**	0.485**
ADM				0.802**	−0.564**	0.789**	0.737**	0.632**	0.826**	−0.879**	0.829**
RDM					NS	0.828**	0.581**	0.656**	0.474**	−0.678**	0.454**
CP						NS	NS	NS	−0.627**	0.519**	−0.641**
NDF							0.646**	0.668**	0.520**	−0.740**	0.576**
ADF								0.635**	0.466**	−0.530**	0.667**
Lignin									NS	−0.475**	0.369*
AZP										−0.861**	0.743**
AZT											−0.745**

CO= crude oil; PJM= young plants in the tillering stage; ADM= aerial dry matter; RDM= root dry matter; CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; AZP= *Azospirillum*; AZT= *Azotobacter*; PSE= *Pseudomonas*.

* $P \leq 0.05$. ** $P \leq 0.01$. NS= non significant.