EFFECTS OF CRUDE OIL ON THE GROWTH OF *Brachiaria mutica* AND *Leucaena leucocephala* AND ON SOIL AND PLANT MACRONUTRIENTS

[EFECTOS DEL PETRÓLEO CRUDO EN EL CRECIMIENTO DE *Brachiaria mutica* Y *Leucaena leucocephala* Y EN LOS MACRONUTRIMENTOS EN SUELO Y PLANTA]

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SUMMARY

This study assessed the effect of 150, 50000 and 78000 mg kg^{-1} of fresh crude oil (FO) and 78456 mg kg^{-1} of weathered crude oil (WO) on the growth of *Brachiaria mutica* and *Leucaena leucocephala*. The variables measured were plant height, dry biomass, nitrogen (N), phosphorus (P) and potassium (K) contents in soil and plant, density of rhizospheric bacteria and fungi, and FO and WO degradation. Statistical differences were found ($P \leq 0.05$) in all variables. *Leucaena leucocephala* was more sensitive to oil toxicity than *B. mutica*. In *L. leucocephala* FO caused the death of the plant from the second month, while WO led to a 6-fold reduction in height. The FO had no effect on biomass accumulation of *B. mutica* but it reduced it in *L. leucocephala*, whereas WO decreased biomass in both species; N, P and K accumulation decreased in *B. mutica* in soil with WO and in *L. leucocephala* in soils with FO and WO. Density of rhizosphere bacteria was inhibited by WO in both species; there were fewer fungi in soil with FO in *B. mutica* and in soil with WO in *L. leucocephala*. Degradation of FO and WO was higher in *B. mutica* rhizosphere soil, so this grass may be an alternative for decontaminating soils polluted with FO and WO.

Key words: Fresh oil; nitrogen; oil degradation; phosphorus; potassium; weathered oil.

RESUMEN

Se evaluó el efecto de 150, 50000 y 78000 mg kg^{-1} de petróleo fresco (PF) y 78456 mg kg^{-1} de petróleo intemperizado (PI) sobre el crecimiento de *Brachiaria mutica* y *Leucaena leucocephala*. Se evaluó altura de la planta, biomasa seca, contenido de nitrógeno (N), fósforo (P) y potasio (K) en suelo y planta, densidad de bacterias y hongos rizosféricos, y degradación de PF y PI. Hubo diferencias estadísticas ($P \leq 0.05$) en todas las variables. *Leucaena leucocephala* fue más sensible a la toxicidad por petróleo que *B. mutica*. En *L. leucocephala* PF causó mortalidad desde el segundo mes, mientras que PI redujo su altura seis veces. El PF no afectó la acumulación de biomasa foliar y radical de *B. mutica* pero la redujo en *L. leucocephala*, mientras que el PI redujo la biomasa en ambas especies. La acumulación de N, P y K disminuyó en *B. mutica* en suelo con PI y en *L. leucocephala* en suelos con PF y PI. La densidad de bacterias rizosféricas fue inhibida por PI en ambas especies; los hongos fueron menos numerosos en suelos con PF en *B. mutica* y con PI en *L. leucocephala*. La degradación de PF y PI fue mayor en suelo rizosférico de *B. mutica*, por lo que este pasto puede ser una alternativa para descontaminar suelos con PF y PI.

Palabras clave: Petróleo fresco; nitrógeno, degradación del petróleo; fósforo; potasio; petróleo intemperizado.

INTRODUCTION

Brachiaria mutica (Para grass) and *Leucaena leucocephala* (White Leadtree) are two native species from tropical regions. Both are important forage-producing species for cattle (Sotomayor-Ríos and Schank, 2001; Wencomo *et al*., 2009), in addition to providing a number of environmental services (SEMARNAT, 2009). *Brachiaria mutica* (Bm) requires waterlogged soils with poor drainage to thrive, and produces up to 20 t ha^{-1} dry matter per year (Farías, 2006). *Leucaena leucocephala* (Ll) is used as a protein source; although it is cultivated in a wide variety of soils, this species prefers deep clayey or loam soils, tolerates draught, adapts to moist sites receiving 2300 mm of rainfall per year, and produces

up to 20 t ha^{-1} dry matter per year (González *et al*., 2003). In Southeast Mexico, Bm is cultivated as pasture for grazing cattle, whereas Ll is used as living fence in livestock pasture lands as well as interspersed with perennial plants in family orchards for human consumption.

Brachiaria mutica has been reported to thrive in soil polluted with weathered crude oil, whereas Ll has been experimentally exposed to crude oil (Rivera-Cruz *et al*., 2005). Plant exposure to oil-polluted soil results in inhibition of growth and biomass production in grasses (Maldonado-Chávez *et al*., 2010; Tang *et al*., 2011) and legumes (Merkl *et al*., 2005; Vázquez-Luna *et al*., 2010). The decrease in plant biomass results indirectly from the difficulty of the plant to adapt to the obstruction of soil pores that causes poor gas exchange, and to a lower water flow, which reduces nutrient supply and availability for the plant (Oluwafemi *et al*., 2008). Nitrogen (N), phosporus (P) and potassium (K) are essential macronutrients for plant growth and development (Bonilla, 2003), and when present in insufficient amounts the plant is subjected to stress. There are plant species which are tolerant to stress derived from the presence of oil in the soil. Some grasses and legumes adapt to these conditions, since the former have a root system with a large surface area for microorganisms, and the latter form roots that host symbiotic atmospheric nitrogen-fixing bacteria (Philippot and Germon, 2005). Oxidation and reduction processes of inorganic and organic chemicals take place in the rizosphere that result in the breakdown of all oil hydrocarbons (Banks *et al*., 2000) and the mineralization of macronutrients such as N (Walecka-Hutchison and Walworth, 2007).

Grasses and legumes growing in soil polluted with hydrocarbons from fresh (FO) and weathered (WO) crude oil have been identified in Mexico's oil-producing regions (García-López *et al*., 2006; Trujillo-Narcía *et al*., 2006; Trujillo-Narcía *et al*., 2011); these plants are capable to decontaminate these soils. However, the potential of these plants to absorb N, P and K and the accumulation of macronutrients in foliar biomass remain unknown, as well as the relationship of oil with soil and plant parameters in tropical areas. Nevertheless, it is affirmed that Bm produces higher amount of biomass, higher N, P, and K buildup in foliar biomass, a lower density of rizosphere bacteria and higher total petroleum hydrocarbons (TPH) breakdown compared with Ll, and that there is lower N, P and K availability in soil contaminated with WO.

Therefore, the aims of this study were to assess the effect of FO and WO on the growth of Bm and Ll, on the density of the rizosphere microflora, on the N, P and K availability in soil, on the N, P and K buildup in the plant, as well as to determine the degradation rate for each type of crude oil.

MATERIALS AND METHODS

Soil

The study included two experiments. Experiment I corresponded to the control group and assessed a mollic Gleysol soil containing 150 mg kg^{-1} TPH of likely biogenic origin, collected from Arroyo Hondo, municipality of Cárdenas, Tabasco, México (17° 59' 50" N and 93° 24' 59" W). Mollic Gleysol is a clay-loam, moderately acidic soil with moderate organic matter content, high P, moderate K and high cation-exchange capacity (NOM-021-RECNAT-2000; DOF, 2002).

Experiment II assessed an anthraquic Gleysol soil (Rivera-Cruz *et al*., 2004) collected at ejido José Narciso Rovirosa, municipality of Huimanguillo, Tabasco, México (18° 04' 54" N and 94° 02' 31" W) in the southern area of La Venta gas-processing complex in Tabasco. This soil has been polluted with oil for 28 years and contains 78456 mg kg^{-1} TPH from WO (WO-TPH); this dose was used as reference to set the higher FO dose in the experiment. Anthraquic Gleysol is clayey, with a high cationic-exchange capacity, according to the procedure by NOM-021-RECNAT-2000 (DOF, 2002), strongly acidic and with a very high organic matter content, low P and moderate K levels (DOF, 2002). Samples from both soil types were collected from the surface horizon (0 to 25 cm) and were dried in the dark at room temperature. Samples were separately fragmented and sieved through a 5 mm metallic sieve (OECD, 2006).

Experiment setup and variables assessed

The two experiments were run for 180 days, each including four treatments and six replicates. Experiments I and II were conducted with Ll and Bm plants, respectively; in both cases, soil moisture was controlled at 30%. *Leucaena leucocephala* seeds and Bm sprouts were planted in conic pots filled with oil-free soil, watered daily and transplanted to definitive pots 40 days after planting. Four treatments were used: T1) mollic Gleysol [150 mg kg^{-1} TPH]; T2) T1+ 50000 mg kg^{-1} TPH from FO [FO-TPH]; T3) T1 + 78000 mg kg^{-1} FO-TPH; and T4) anthraquic Gleysol (78456 mg kg^{-1} WO-TPH). The variables assessed were the following: plant growth, accumulation of N, P and K both in soil and in the plant, microflora density and degradation rate for each oil type.

The T4 treatment (anthraquic Gleysol) was included because WO spills occur frequently in Tabasco's oil fields; this was contrasted with T3, having a similar

FO concentration, in order to investigate whether the variables assessed respond differently according to oil type (FO or WO).

The FO was obtained from the Cinco Presidentes oil field, having 29.7° API and a density of 0.867 g cm^{-3} at 40 °C. On the other hand, the WO was oil accumulated during 28 years in soil from oil spills and leaks, which has been exposed to environmental factors.

Chemical analysis in soil and plants

The TPH were extracted from soil at baseline (day 1) and when the experiment was completed (day 180), to determine the percentage of degradation. The extraction was conducted using 99.5% dichloromethane (Merck®) in a soxhlet apparatus following the EPA method 418.1 modified for soil and sediment (EPA, 1986). The TPH were determined gravimetrically according to the procedure by NMX-AA-134-SCFI-2006 (DOF, 2006). Chemical analyses in soil and plants were conducted in samples collected at the end of the study. Total N in soil was determined using the micro-Kjeldhal method (Page *et al*., 1982); available P was measured through the Olsen method using a $NaHCO_3$ extraction solution and read in a UV-visible spectrophotometer at 882 nm (Olsen and Sommers, 1982); available K was determined in a flame photometer using neutral $C_2H_4O_2 \cdot H_3N$ (Jackson, 1973). Plants were washed with distilled water and dried at 72 °C for 72 h, ground and sieved. Total N in the plant was extracted by digestion with HNO_3 (Page *et al*., 1982) and quantified using the micro-Kjeldhal method (Jones *et al*., 1992); total K in the plant was measured in an atomic absorption spectrophotometer, and total P was extracted with 3 mL H_2SO_4 and 1 mL H_2O_2 at 360 °C and quantified with the Berthelot reaction and molybdenum blue method (Alcántar and Sandoval, 1999).

Microbiological testing

The density of heterotrophic bacteria and fungi (CFU g^{-1} dry soil) was determined using the serial dilution agar-plate technique (Madigan *et al*., 2009). Ten grams of sample were used, preparing serial decimal dilutions; a 0.1 mL-aliquot of solution was dispersed per Petri dish in culture medium selective for heterotrophic bacteria (nutrient agar) and for heterotrophic fungi (potato dextrose agar). Plates were incubated at 28 °C for 48 h for bacteria and 72 h for fungi.

Plant growth and biomass

Plant height was measured with a ruler graduated in mm at 30-day intervals for six months, from the base of the stem to the leave bud. Plants were harvested six months after planting (day 180), roots were separated from shoots, washed with water, placed into paper bags and oven-dried at 72 °C for 72 h. Root and shoot dry biomass was determined by weighing in a semi-analytical balance with a precision of 0.01 g. Total dry biomass was obtained by adding up shoot and root dry biomass.

Statistical analyses

Analysis of variance, separation of means (Tukey, $P \leq 0.05$) and correlation analysis of soil and plant variables were conducted with the GLM procedure of the software SAS for Windows ver. 8.1 (SAS Institute Inc., 2000). Soil variables were densities of heterotrophic bacteria and fungi, total N, available P, available K and oil degradation. Plant variables assessed were height, root dry biomass, foliar dry biomass, total dry biomass and total foliar N, P and K content.

RESULTS AND DISCUSSION

Soil macronutrients and microflora

No statistical differences ($P \leq 0.05$) were found in total N content in soil between the control soil and the soils polluted with 50000 and 78000 mg kg^{-1} FO, but a lower amount of total N ($P \leq 0.05$) was found as a result of the effect of 78456 mg kg^{-1} WO in both soil types in which Bm and Ll were grown (Table 1). Total N levels in soil where Bm was planted were 0.36, 0.35, 0.32 and 0.30% for control soil, soil with 50000 mg kg^{-1} FO-TPH, soil with 78000 mg kg^{-1} FO-TPH, and soil with 78456 mg kg^{-1} WO-TPH, respectively. Coefficients of variation were up to four times as high as the average in soils with 50000 and 78000 mg kg^{-1} FO (Table 1), probably because of oil redistribution by water runoff during irrigation. Many microorganisms synthesize and release urease, the enzyme responsible for the hydrolysis of urea (Alexander, 1994), and it is likely that because of this biochemical feature total N was similar in the control soil and in those with pollution levels up to 78000 mg FO.

Available P in soil also showed differences between treatments in both experiments as well as in both oil types. In soil with WO, P availability decreased 12 and 3.5 times relative to soil with 78000 mg FO in which Bm and Ll were grown, respectively (Table 1). This response suggests that the soil solution contains a lower amount of available P compared to the soil with FO or to the control soil containing 150 mg TPH. Available K in the soil solution displayed a positive relationship with FO but decreased as an effect of WO in both experiments (Table 1). Increasing FO levels promoted K availability; this response appears to be

related to a higher K mineralization rate from organic or mineral substrates. Regarding the inhibition of K availability in soil with WO, it has been reported that the recalcitrant property of weathered crude oil negatively influences mineralization (Porta *et al*., 2003). Results of Wang *et al*. (2009) reveal decreasing trends in the amount of total N and available P in soil polluted with oil for 5, 10 and 20 years; N content in these years was 1.17, 0.67 and 0.84 g kg^{-1}, and the corresponding values for P were 0.37, 0.32 and 0.26 g kg^{-1}, respectively. This response may be associated with the density of microorganisms in the three soils (Mohn and Stewart, 2000).

Table 1. Total N, available P and available K in soil polluted with fresh and weathered oil, in which *Brachiaria mutica* and *Leucaena leucocephala* were grown. Samples collected at 180 days after planting.

TPH (mg kg^{-1} dry weight)	*Brachiaria mutica*			*Leucaena leucocephala*		
	Total N (%)	Available P (mg kg^{-1})	Available K	Total N (%)	Available P (mg kg^{-1})	Available K
150 PB[¶]	0.36 ± 0.1^{a}	20.7 ± 1.7^{b}	24 ± 1.0^{b}	0.34 ± 1^{a}	23 ± 1.2^{a}	36 ± 0.05^{ab}
50,000 FO[§]	0.35 ± 1.4^{a}	26 ± 1.4^{b}	27 ± 1.6^{b}	0.31 ± 1.1^{a}	24 ± 2^{a}	36 ± 1.03^{ab}
78,000 FO	0.36 ± 0.8^{a}	32 ± 1.1^{a}	42 ± 1.5^{a}	0.34 ± 1.1^{a}	24 ± 4.3^{a}	49 ± 0.16^{a}
78,456 WO[‡]	0.30 ± 0.6^{b}	2.7 ± 2^{c}	28 ± 0.5^{b}	0.27 ± 0.6^{b}	7 ± 1^{b}	27 ± 0.2^{b}

[¶]Likely biogenic oil, [§]fresh oil and [‡]weathered oil.
[a,b,c]Means with a different superscript are statistically different (Tukey, $P \leq 0.05$, a>b, n=6).

The data obtained in this investigation confirm the positive effect of rizosphere bacteria on total N and available P in the presence of Bm, and only on available P in the association of bacteria with Ll (Table 2).

Table 2. Pearson's correlation between *Brachiaria mutica* and *Leucaena leucocephala* variables, soil microorganisms and macronutrients in samples collected on day 180 after planting.

Species/ variable	Plant height	Total Biomass	Density		Nutrients in soil			Nutrients in plant		
			Fungi	Bacteria	Total N	Avail. P	Avail. K	N	P	K
Brachiaria										
TPH-degradat	-.654*	-.748**	-.624*	NS***	NS	NS	NS	-.919**	.732**	.831**
Plant height		.964**	.601*	.615*	.764**	NS	NS	NS	NS	NS
Total biomass			.651*	NS	.665*	NS	NS	.622*	NS	NS
Dens. fungi				NS	NS	NS	NS	NS	NS	NS
Dens. bacteria					.907**	.817**	NS	NS	.597*	NS
N-soil						.705*	NS	NS	NS	NS
P-soil							NS	NS	.716**	NS
K-soil								NS	NS	NS
N-plant									-.680*	-.835**
P-plant										.678*
Leucaena										
TPH-degradat	NS	NS	NS	.661*	NS	NS	NS	-.593*	-.594*	NS
Plant height		.990**	NS	NS	NS	NS	NS	.736**	.843**	.841**
Total biomass			NS	NS	NS	NS	NS	.636*	.763**	.803**
Dens. fungi				.578*	NS	NS	NS	-.615*	-.603*	NS
Dens. bacteria					NS	.807**	-.618*	-.868**	-.781**	NS
N-soil						.670*	NS	NS	NS	NS
P-soil							-.866**	NS	NS	NS
K-soil								NS	NS	NS
N-plant									.971**	.831**
P-plant										.881**

*Correlation is significant at $P \leq 0.05$ and **at $P \leq 0.01$, n=12. ***NS: no significance.

The average density of heterotrophic bacteria and fungi in Bm and Ll rizosphere soil revealed statistically significant differences ($P \leq 0.05$) (Table 3). The highest population densities of both fungi and bacteria associated with these two plant species corresponded to oil-free soil, and the lowest figures were recorded in soil containing 78456 mg kg^{-1} WO. This response is likely related to the lower amount of air in soil, and also to the decrease in moisture retention which restrains aerobic microbial reproduction, given that highly compacted aggregates tend to occur because of the resin and asphaltene content associated with WO in soil (Delʿ Arco and França, 2001).

Table 3. Density of heterotrophic bacteria and fungi in *Brachiaria mutica* and *Leucaena leucocephala* rizospheres. Samples collected on day 180 after planting.

TPH (mg kg^{-1} dry weight)	*Brachiaria mutica*		*Leucaena leucocephala*	
	Bacteria	Fungi	Bacteria	Fungi
	CFU per gram dry rizosphere			
150 PB¶	$1100x10^4 \pm 649x10^{4\ a}$	430 ± 685^a	$200x10^4 \pm 169x10^{4\ a}$	$10x10^2 \pm 14x10^{2\ a}$
50,000 FO§	$900x10^4 \pm 585x10^{4\ b}$	76 ± 44^c	$160x10^4 \pm 127x10^{4\ b}$	$14x10^2 \pm 10x10^{2\ b}$
78,000 FO	$161x10^4 \pm 34x10^{4\ c}$	70 ± 65^c	$99x10^4 \pm 69x10^{4\ c}$	$19x10^2 \pm 18x10^{2\ c}$
78,456 WO‡	$10x10^4 \pm 8x10^{4\ c}$	110 ± 89^b	$31x10^4 \pm 34x10^{4\ d}$	$9x10^2 \pm 7x10^{2\ d}$

¶Likely biogenic oil, §fresh oil and ‡weathered oil.
[a,b,c]Means with a different superscript are statistically different (Tukey, $P \leq 0.05$, a>b, n=6).

Plant growth, biomass and nutrient buildup

In Bm and Ll, plant height at 1 month was similar across treatments ($P \leq 0.05$; Table 4). This lack of response was likely due to the fact that the root was still adapting to the stressing conditions in soil, as well as because the root of the transplanted seedling was still surrounded by oil-free soil where it grew in the seedbed. Statistical differences ($P \leq 0.05$) were evident from month 2 (Table 4). Through time, plant growth in Bm was inversely related to FO dose; however, plant growth was similar in soil with WO and in soil with 78000 mg kg^{-1} FO after two, three and four months ($P \leq 0.05$), and differed after five and six months. Two and three months after transplanting Ll in soil with 50000 and 78000 mg kg^{-1} FO, plants dried out completely; however, plants became adapted to 78456 mg WO, although plant height was six times smaller compared to plants grown in the control soil. This toxic effect was likely caused by the light and medium fractions contained in fresh oil, which according to Freedman (1989), when in contact with the plant kill the foliage and some woody tissues, since these form an impermeable coating similar to wax around soil aggregates, restraining water retention and, hence, water availability for the plant. Consequently, according to Li *et al*. (1997), the efficiency of the plant regarding water use decreases, and plant growth may be seriously affected. However, not all perennial tissues are damaged by oil to the extent of being necrotized. Such was the case of Bm, which displayed tolerance to FO, likely as a result of root vigor (Walton *et al*., 1994). According to Banks *et al*. (2000), grasses have fibrous root systems which provide a broad surface area for rizosphere microorganisms, so that the plant becomes more stress-tolerant and can maintain its productivity.

The production of dry biomass of root and leaf of Bm was similar ($P \leq 0.05$) in control treatments and in 50000 and 78000 mg of FO, but biomass in plants established in soil with 78456 mg of WO was different (Figure 1). As for Ll, the results show that FO caused decreased root and leaf biomass and WO caused less reduction in biomass. Foliar biomass of Bm compared with the control increased 2.9% in soil with 50000 mg FO. These results agree with Lindau and Delaune (2000), who found that petroleum hydrocarbons increased biomass and stem density of *Spartina lancifolia* with respect to the control site. The biomass of Bm decreased 6.1 and 63.5% by effect of exposure to 78000 mg kg^{-1} FO and WO, respectively. Similar results were obtained by Rivera-Cruz *et al*. (2004) in grass (*Echinochloa polystachya*) by the effect of 50000 and 100000 mg kg^{-1} FO, where dry matter was reduced 51 and 53%, respectively. The root and foliar biomass of Ll were not harvested because the plants died from the effect of the second month of the doses of 50000 and 78000 mg kg^{-1} FO. The dose of 78456 mg kg^{-1} WO resulted in the reduction of 94.7 and 98.3% of roots and leaves, respectively (Figure 1).

The accumulation of N, P and K in the foliage of Bm and Ll showed significant differences ($P \leq 0.05$; Table 5) as a result of exposure to FO and WO for 180 days. A highly significant relationship was observed between plant height and biomass and N, P and K buildup in the foliage of both species (Table 2). The highest nutrient accumulation occurred in both species

grown in oil-free soil. In Bm, N and P buildup was unaffected by FO, but K accumulation dropped as an effect of 78456 mg kg^{-1} WO. The FO caused toxic effects on Ll, and both plant species displayed a negative effect on the accumulation of the three macronutrients as a result of the presence of WO in soil (Tables 2 and 5).

Table 4. Plant height in *Brachiaria mutica* and *Leucaena leucocephala* in soil polluted with fresh and weathered soil at six different time points.

TPH (mg kg^{-1} dry weight)	Month					
	1	2	3	4	5	6
	cm					
Brachiaria						
150 PB¶	30.3 ± 4.5[a]	87 ± 14.1[a]	127.7 ± 29.6[a]	166.8 ± 33.9[a]	194.6 ± 37.8[a]	218 ± 34.9[a]
50,000 FO§	29.7 ± 3.6[a]	70.7 ± 8.2[ab]	91.7 ± 8.1[b]	111 ± 15.8[b]	136 ± 10.9[b]	153 ± 14[b]
78,000FO	28.8 ± 4.9[a]	53 ± 11.7[b]	72.7 ± 14.1[b]	98 ± 17.6[b]	110.3 ± 13.5[bc]	130.7 ± 11.9[bc]
78,456 WO‡	30.7 ± 8.6[a]	63 ± 11.9[b]	86 ± 18.6[b]	95.3 ± 22.9[b]	101.7 ± 22.8[c]	110.3 ± 23.7[c]
Leucaena						
150 PB	10.7 ± 1.9[a]	20.8 ± 7.1[a]	42.8 ± 13.7[a]	68 ± 19.6[a]	104.3 ± 27.9[a]	142.3 ± 25.3[a]
50,000 FO	11.8 ± 1.8[a]	13.3 ± 1.7[ab]	17 ± 3.9[b]	0[c]	0[c]	0[c]
78,000FO	9.2 ± 1.7[a]	0[c]	0[c]	0[c]	0[c]	0[c]
78,456 WO	9.8 ± 0.6[a]	10.6 ± 1.3[b]	12.9 ± 1.7[b]	15.2 ± 4.6[b]	20 ± 8.1[b]	23.8 ± 11.2[b]

¶Likely biogenic oil, §fresh oil and ‡weathered oil.
[a,b,c]Means with a different superscript are statistically different (Tukey, $P \leq 0.05$, a>b, n=6).

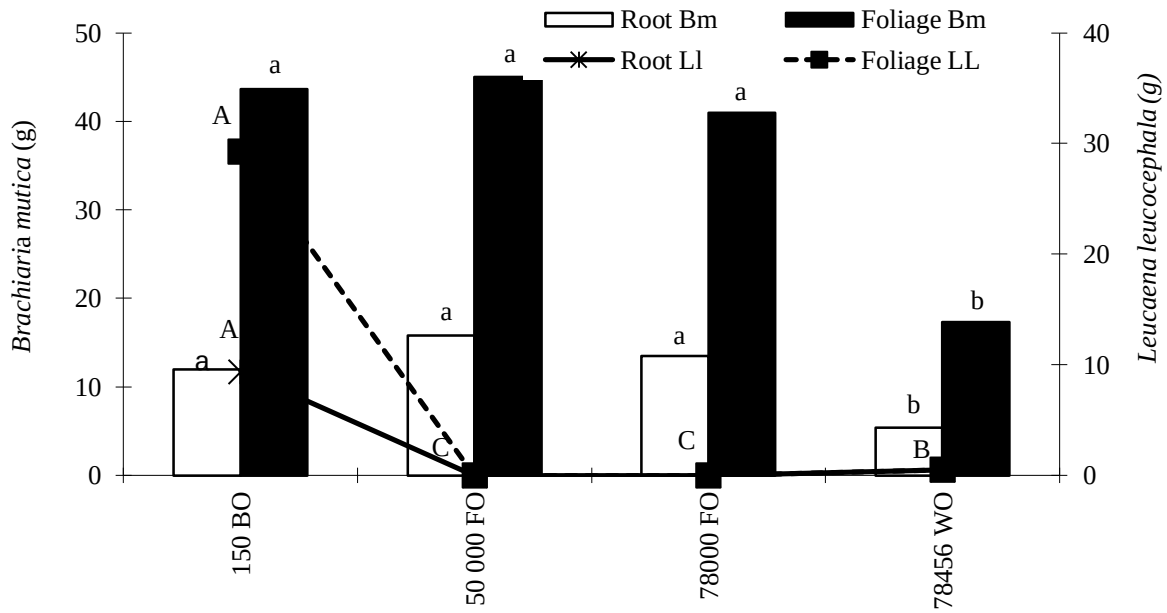

Figure 1. Effect of fresh and weathered oil on root and foliar biomass in *Bracharia mutica* and *Leucaena leucocephala* on Day 180 after transplanting.
Figures with different small letters in each column and different capital letters between points connected by solid lines mean statistically significant differences between treatment means (Tukey´s $P \leq 0.05$, a>b, n=6).

Tabla 5. Dose effect of oil present in soil on buildup of three macronutrients in foliar biomass of samples collected on day 180 after planting.

TPH (mg kg^{-1} dry weight)	*Brachiaria mutica*			*Leucaena leucocephala*		
	N	P	K	N	P	K
			%			
150 PB[¶]	0.91 ± 0.3^{ab}	1.57 ± 0.67^{a}	0.12 ± 0.02^{a}	0.5 ± 0.18^{a}	1.1 ± 0.4^{a}	0.96 ± 0.3^{a}
50,000 FO[§]	1.09 ± 0.21^{a}	1.36 ± 0.42^{a}	0.2 ± 0.05^{a}	0^{b}	0^{b}	0^{b}
78,000 FO	1.19 ± 0.16^{a}	1.29 ± 0.41^{a}	0.11 ± 0.05^{b}	0^{b}	0^{b}	0^{b}
78,456 WO	0.55 ± 0.2^{c}	0.37 ± 0.13^{b}	0.04 ± 0.02^{c}	0.02 ± 0.11^{b}	0.01^{b}	0.01^{b}

[¶]Likely biogenic oil, [§]fresh oil and [‡]weathered oil.
[a,b,c]Means with a different superscript are statistically different (Tukey, $P \leq 0.05$, a>b, n=6).

Oil hydrocarbon degradation

The removal of FO-TPH and 78456 mg kg^{-1} of WO was stimulated by both plant species. Significant differences were observed between plant species as well as between oil concentrations in soil ($P \leq 0.05$). *Brachiaria mutica* induced the highest degradation (55%) in soil containing 50000 mg kg^{-1} FO after 120 days; however, when exposed to 78000 mg FO and 78456 mg kg^{-1} WO degradation dropped to 41.3 and 27.6%, respectively (Figure 2). The greater efficiency of grasses in soil restoration was previously reported by Rivera-Cruz *et al*. (2004) and Merkl *et al.* (2005), who pointed out that grasses are effective for this because they produce a dense network of roots reaching as far as 2.7 m deep in soil (Gould and Shaw, 1997), secreting exudates which contribute to the proliferation of microorganisms that are key for the breakdown of soil pollutants, leading to the neutralization of their toxic properties (Davis *et al*., 2002).

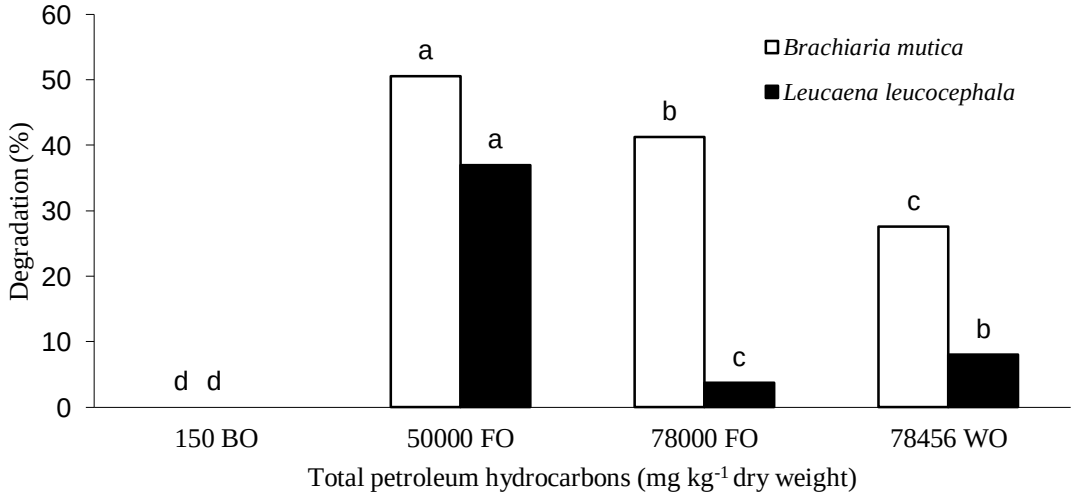

Figure 2. Average degradation of total petroleum hydrocarbons in soil with biogenic oil (BO), fresh oil (FO) and weathered oil (WO) in which *Brachiaria mutica* and *Leucaena leucocephala* were grown, 180 days after planting (Tukey $P \leq 0.05$, a>b, n=6).

In this study, the type of oil proved to be a determining factor in the percentage of TPH removal. In the presence of Bm and at 78000 mg kg^{-1} FO a higher degradation rate and volatilization occurred relative to hydrocarbons derived from 78458 mg kg^{-1} WO. This response is likely due to that WO has a higher proportion of high-molecular weight (MW) hydrocarbons such as C20 to C40 alkanes, polynuclear aromatic hydrocarbons (PAHs), asphaltenes and resins, which are degraded slowly. According to Huesemann (2004), the persistence of these oil fractions is explained by their low solubility, and

hence these require long treatments involving sequential processes. On the other hand, FO hydrocarbons contain the hydrocarbons mentioned above plus the fraction of low (C5-C9) and medium (C10-C18) MW n-alkanes and aromatic hydrocarbons that are volatilized and/or degraded relatively easy. Another factor that affects oil degradation rate is soil humidity. Biodegradation is much slower under anaerobic conditions than in the presence of oxygen (Atlas and Bartha, 2002).

Relationship between biological and chemical parameters

For Bm, foliar and total biomass evidenced a high correlation with soil N, P and K levels. The fertility level promoted plant growth, so that it was also correlated with foliar content (Table 2). However, Ll did not display this same relationship with soil macronutrients, but it did in the case of nutrient buildup in foliage (Table 2). The high sensitivity of this leguminous species to FO in the soil limited its growth, contrary to the result when Ll was grown in soil with WO; however, both productivity and N, P and K buildup in foliage dropped. The percentage of TPH degradation in soil displayed a significant negative correlation with fungus density ($r = -0.624$), but not with heterotrophic bacteria density in Bm rizosphere; in the Ll rizosphere, TPH degradation was positively correlated ($r = 0.661$) only with bacterial density (Table 2). Maldonado-Chávez *et al*. (2010) reported similar findings between microbial consortia and oil hydrocarbon degradation.

The grass Bm constitutes an option suitable to be cultivated in soils polluted with FO and WO, because it stimulates decontamination and has the potential to produce forage in oil-producing zones. However, further studies are required to investigate whether this plant species accumulates hydrocarbons, which would affect the quality of livestock production.

CONCLUSION

Doses of 50000 and 78000 mg kg^{-1} FO-TPH caused the death of Ll; however, this legume survived at 78456 mg kg^{-1} WO-TPH. *Brachiaria mutica* survived at all TPH doses, achieving different heights as a result of the effect of dose and type of FO- and WO-TPH. The dose of 50000 mg kg^{-1} FO stimulated plant biomass production.

The Bm rizosphere induced the best response to the effect of the two FO doses and the WO dose on total N and available P and K in soil and accumulated in foliage. This suggests a physiological adaptation ability of this plant species to oil-polluted soils.

The positive relationship of plant height and foliar biomass production in Bm with density of heterotrophic bacteria and fungi in rhizospheric soil, with macronutrient content in soil and with those accumulated in foliage are all positive indicators of adaptation to pollution.

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