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Tolerance of Four Tropical Tree Species to Heavy Petroleum Contamination

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Abstract Four species of trees were selected to evaluate the tolerance to heavy crude oil contamination by means of a tolerance index integrating germination, height, biomass and survival as variables. Fresh seeds to *Cedrela odorata* (tropical cedar), *Haematoxylum campechianum* (tinto bush), *Swietenia macrophylla* (mahogany) and *Tabebuia rosea* (macuilis) were planted in a Vertisol to which heavy crude petroleum was added at four different treatments (C0, 0; C1, 18,940; C2, 44,000; and C3, 57,000 mg kg^{-1}), with the control being uncontaminated soil. The experiment was carried out in a greenhouse during 203 days with a completely random design. The presence of petroleum in soil stimulated and increased germination of *S. macrophylla* and *C. odorata*, accelerated the germination of *T. rosea* and did not affect the germination of *H. campechianum*. The height and biomass of all species was reduced in the presence of petroleum in the soil. The survival of *S. macrophylla* and *H. campechianum* was not affected by petroleum at any concentration studied. On the other hand, *C. odorata* and *T. rosea* showed high mortality at all concentrations. The tolerance index showed that *S. macrophylla* was best at tolerating petroleum in soil and could be employed as a productive alternative for the advantageous use of contaminated sites. The use of tree species could be important because of the great potential of trees for phytoremediation due to their long life, biomass and deep roots that can penetrate and remediate deeper soil layers.

Keywords Phytoremediation · Microorganisms · Oxidative stress · Pollution · Toxicity

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1 Introduction

Petroleum is one of the dominant sources of energy worldwide. The use of the fuels contributes largely to the economic and social development of a country

(White et al. 2006; Mohsenzade et al. 2009). The large-scale development of the petroleum industry has resulted in soil, water and air contamination, which is a problem that has not yet been able to be avoided. The major causes of oil pollution are spills during extraction and transport of crude petroleum and its derivatives (Bossert and Bartha 1984; Saval 1997). Soils with petroleum hydrocarbons in high concentrations can represent a risk to public health and the environment (Mohsenzade et al. 2009).

When a spill of crude oil or its derivatives occurs, immediate harm to flora and fauna as well as to site microorganisms is caused (Freedman 1995), due to the potential toxicity of some of the petroleum hydrocarbons (Pothuluri and Cerniglia 1994). The toxicity depends on the quantity spilled, type of petroleum, exposure time, environmental factors and sensitivity of the organisms in the affected ecosystem (Adam and Duncan 2002; Merkl et al. 2005). In plants, the toxic effects of petroleum are manifested as foliage and tissue necrosis, decreased production of photosynthetic pigments, reduction in the aerial and root biomass, necrosis and even death (Chaineau et al. 1997; Adam and Duncan 2002).

The expression of toxicity caused by total petroleum hydrocarbons (TPH) and the tolerance to these compounds is variable in the vegetable kingdom, even among members of the same botanical genus (Adam and Duncan 2002; Quiñones Aguilar et al. 2003). The vegetative species that have a capacity to grow in TPH-contaminated soils have been used in recent years for bioassays to identify the degree of tolerance and capacity to degrade petroleum hydrocarbons and to be used in the process known as phytoremediation (Cunningham et al. 1996; Salt et al. 1998). Phytoremediation has been defined as a technology that uses green plants, rhizospheric microorganisms and agronomic techniques to degrade contain or transform the organic or inorganic contaminants to less toxic or less mobile compounds (Cuhhingham et al. 1996; Pilon-Smits 2005).

To identify and select species with the capacity to grow in petroleum hydrocarbon-contaminated soils, tests are frequently run on germination and growth of plantlets in different concentration and types of petroleum hydrocarbons (Rivera-Cruz et al. 2004; Merkl et al. 2006; Besalatpour et al. 2008; Shirdam et al. 2008; Inckot et al. 2011). Another area of interest is the identification of sensitive species to use as bioindicators of the toxicity of a contaminant in soil (Salanitro et al. 1997; Chaineau et al. 2003; Rivera-Cruz et al. 2004; Dawson et al. 2007; Vázquez-Luna et al. 2010).

In the present study four species of trees were selected with the objective of evaluating their tolerance to soil contaminated with heavy crude petroleum by using the variables of germination, growth (height and biomass) and survival. The trees used in this experiment are species native to the humid tropics in Mesoamerica. These species were selected because they are found to be naturally established in soils contaminated with petroleum and because they are multiple use species (wood, live fence, supports, shade for cattle, fuel) in the rural communities in Tabasco, Mexico (Ochoa-Gaona et al. 2011). There are no previous experimental studies on the resistance of these species to petroleum hydrocarbon-contaminated soil. The species which show the best results in this investigation could be used for studies on phytoremediation and possibly be used as a productive alternative for petroleum-contaminated soils, which generally are characterized as having low productivity.

2 Materials and Methods

For this experiment, recently collected seeds of *Cedrela odorata* (tropical cedar), *Haematoxylum campechianum* (tinto bush), *Swietenia macrophylla* (mahogany) and *Tabebuia rosea* (macuilis) were used. A heavy crude petroleum was used from southern Veracruz state and uncontaminated soil from an alluvial plain in western Tabasco state.

2.1 Soil Characteristics

The soil used in this study was collected from the surface (0–30 cm, A horizon) of a Vertisol near the Sánchez Magallanes oil field, Cárdenas, Tabasco in SE Mexico (18°06′05.74″ N, 93°52′59.58″ W), but from an uncontaminated area. The sample was air dried in the shade and manually ground to pass through a 5-mm screen. This soil presented a gilgai micro-relief in the field and a clayey texture, typical of Vertisol, with high soil organic matter content (10.4 %). The properties of the petroleum and soil are presented in Table 1.

2.2 Crude Petroleum Characteristics

A heavy crude petroleum (12.9° API) was obtained from an inactive sulphur well in Texistepec, Veracruz

Table 1 Physical and chemical characteristics of soil and crude petroleum used in the tolerance experiment on trees exposed to heavy crude petroleum

Soil characteristics		Crude oil characteristics	
Sand	9 %	API gravity	12.9°
Silt	41 %	Specific gravity	0.98 g cm^{-3}
Clay	50 %		
Texture	Silty clay	Group fractions[a]	
pH	7.2	Aliphatic	37.9 %
SOM	10.4 %	Aromatic	22.0 %
Total N	0.52 %	Polars+resins	20.3 %
Total P	2.35 mg kg^{-1}	Asphaltenes	19.8 %
Hydrocarbon concentration (biogenic origin)[b]	240 mg kg^{-1}		

[a]Determined as per Díaz-Ramírez (2004)

[b]Determined by Soxhlet extraction, US EPA-3540C (1996)

(17°54′20.93″ N, 94°48′16.07″ W). This oil was used due to the similarity that one would expect with the oil in older spills, after a prolonged period of natural attenuation and the resultant weathering. It was enriched in heavier components, being over 40 % asphaltenes, polars and resins, and with a density only slightly less than water.

2.3 Design and Experimental Setup

The experiment was run in a greenhouse under semi-controlled conditions (fiber glass sheet roofing and siding, with 50 % shade screen). The average temperature during the experiment was 30 °C with an ambient humidity of 39.6 % (89.9 maximum, 22.4 % minimum). The greenhouse was located 10 m above sea level in Tabasco, SE Mexico, at coordinates (17°54′41.58″ N, 93°02′10.21″ W). The climate in the area is wet and hot with abundant rain in the summer, an average temperature of 27 °C and average annual precipitation of 2,200 mm (INEGI 2005). The test was run for 203 days from 9 May to 30 November, 2011. The experiment was conducted using a completely random design with a 4×4 factorial arrangement with four different contamination levels of heavy petroleum and four tree species. The heavy petroleum was manually mixed with the soil until it was homogenous; then let set under a roofed area at environmental temperature for 5 days. Subsequently, five samples of soil for each treatment (C0, 0, control; C1, 18,940; C2, 44,000; and C3, 57,000 mg kg^{-1}) were collected to determine the TPH content using dichlormethylene as a solvent (Soxhlet method, US EPA-3540C 1996, Table 2). The soil, thus contaminated with each treatment, was deposited in experimental units of 30×30×25 cm deep with a capacity of 3.5 kg of soil each. Each experimental unit was planted with 30 seeds of the same species, with four replicates per treatment and per species. A pre-germination treatment was given to the seeds of each species which consisted of submerging them in water for 12 h prior to planting (Mulawarman et al. 2003). To facilitate the planting of *H. campechianum* seeds, the fruits were cut in half and placed vertically in the soil. Potable water was added to the soil to maintain it at 30 % total humidity.

2.4 Variables Evaluated

Every 8 days the following variables were registered:

Seeds germinated	was monitored for 48 h after planting.
Plant height	As soon as the seed germinated, the height was measured from the base of the plantlet to the highest terminal bud.
Plant survival	The total of germinated seeds was considered as 100 % of live plantlets, quantity from which was subtracted the percentage of dead plants during the experiment.

Table 2 Total petroleum hydrocarbon content in the four treatment applied to soil, (Soxhlet, US EPA-3540C, 1996)

Treatments	TPH content (mg kg^{-1})
C0 (control)	240[a]
C1	18,940
C2	44,000
C3	57,500

[a] Hydrocarbons of biogenic origin

Total biomass (dry, aerial and root)	At the end of the experiment, each experimental unit was sampled, selecting ten plants at random, for each species. These were dried in an electric oven at 70 °C for 3 days.

The index proposed by Porta et al. (1999) was adapted to calculate the tolerance of each species for soil treatment. The following equations were used:

$$TIcx = 1 - [(TIV_1 + TIV_2 \ldots + TIV_n)/m], \text{ wherein } = 1, 2, 3\ldots$$

$$TIV_n = (C_pR_1 + C_pR_2.. + C_pR_n) / (CcR_1 + CcR_2.. + CcR_n), \text{wherein } = 1, 2, 3\ldots$$

Where: $TIcx$ is the tolerance index (TI) for treatment x and species x, TIV_n is the tolerance index for variable n, m is the number of variables, C_p is the treatment with petroleum, R_i is the replicate, Cc is the control. When: TI=0, the TPH concentration does not affect the vegetable species, TI <0, the TPH concentration stimulates the vegetable species, TI >0, the TPH concentration causes negative effects on the vegetable species. Greater TI values, the species are more affected from the doses of petroleum in soil.

2.5 Statistical Analysis

The data from the variables studied were evaluated by applying a Kruskal–Wallis analysis of variance test to determine the differences between the treatments applied to the soil. The differences between the means were established using a Mann–Whitney procedure. The SPSS statistical package (version 10) was used to analyse the data. For *C. odorata* and *T. rosea* the experiment was concluded at 88 days (30 August, 2011) due to the fact that these two species presented a high mortality rate before the experiment was finalized.

3 Results

3.1 Germination

The germination percentage of *C. odorata*, *H. campechianum* and *T. rosea* in the soil, at all TPH concentrations, was not significantly different (P<0.05) from that in soil without petroleum (C0). The change in germination rate was significantly greater (P<0.05) for *S. macrophylla* in the soil with petroleum (C1=83 %, C2=80 %, C3=84.2 %) in relation to the control (47.5 %). Sixteen days after planting seeds of this species, and until the end of the experiment, the differences were significant. For *T. rosea*, 8 days after the beginning of the experiment, the soil with petroleum had a significantly greater quantity of germinated seeds. However, at 16 days, and at the end of the experiment, there were no significant differences.

The species with the greatest quantity of seeds germinated was *T. rosea* (C2=94 %, C3=98.3 %), followed by *H. campechianum* (C1=79.1 %, C3=72.5 %). The species with the lowest quantity of seeds germinated was *C. odorata* with values between 57.5 % (C0) and 79 % (C2, Fig. 1).

3.2 Plant Growth

3.2.1 Height

At the end of the experiment, all species were significantly taller in the soil without petroleum than in the contaminated soil (P<0.5). The final height *of C. odorata*, *H. campechianum* and *T. rosea* were less in C2 and C3 than in C1 (P<0.5). *S. macrophylla* did not present significant differences (P<0.5) between the treatments with petroleum.

After day 16, and until the end of the experiment, *C. odorata* and *H. campechianum* showed greater height (P<0.05) in the C0 treatment, than in the treatments with petroleum. For *T. rosea*, this was also observed after day 24. On the other hand, *S. macrophylla* showed a significantly greater (P<0.05) height in the treatments with petroleum than in the uncontaminated control at the beginning and up to day 24 of the experiment. Later there were no significant differences (P<0.05) up to day 80, and then the uncontaminated control (C0) showed the greatest height, which was significant (P<0.05). For all species the height was greater in C0 than in C3 (Fig. 2).

3.2.2 Biomass

The quantity of biomass in all of the species studied was significantly greater in the C0 treatment than in the treatments with petroleum (P<0.05). The biomass in *H. campechianum* and *S. macrophylla* did not show

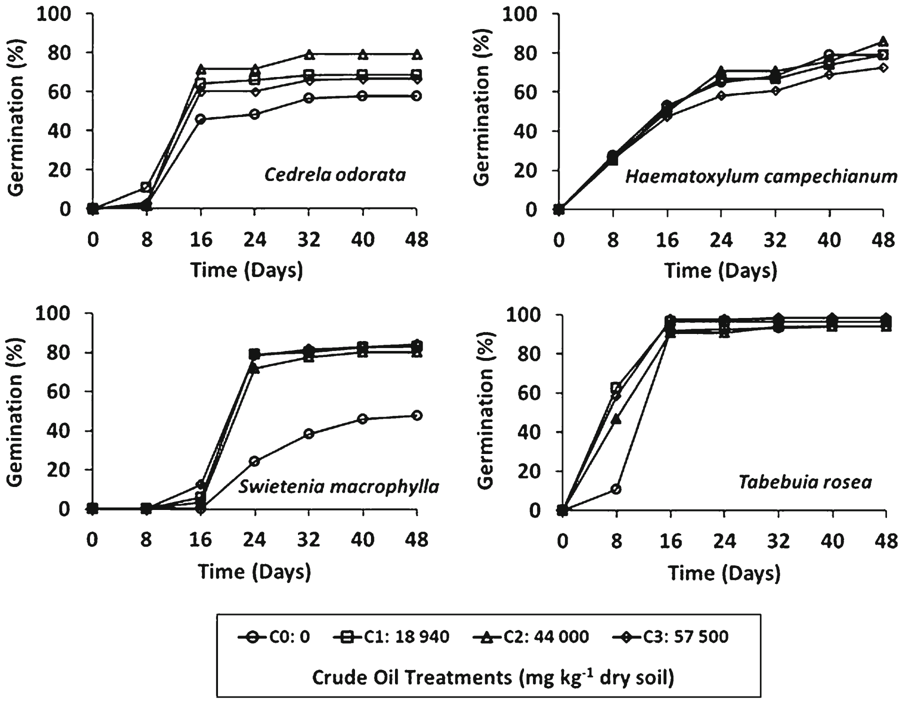


Fig. 1 Seeds germinated (mean percent) for four species of trees planted in soil with four treatments of heavy petroleum

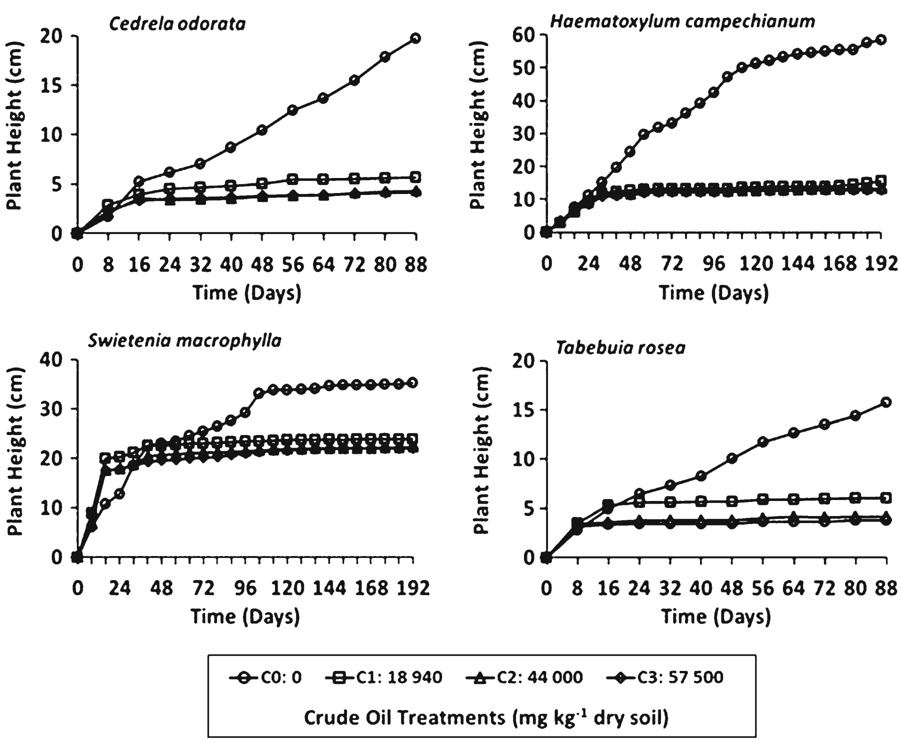


Fig. 2 Average height of four species of trees germinated in soil with four different treatments of heavy petroleum

significant differences between the various treatments with petroleum ($P<0.05$). For *C. odorata*, a significantly difference was found between the biomass in treatment C1 in comparison to treatments C2 and C3 ($P<0.05$). *T. rosea* showed greater biomass in treatments C1 and C2 with respect to C3 ($P<0.05$, Fig. 3). The biomass in the treatments with petroleum in comparison with treatment C0 was reduced up to 97 % for *C. odorata* (C3), 84 % for *H. campechianum* (C3), 73 % for *S. macrophylla* (C2), and for *T. rosea*, up to 96 % (C3).

3.2.3 Plant Survival

The survival of *H. campechianum* and *S. macrophylla* was not significantly different between treatments and the control ($P<0.05$). These two species showed the highest survival rates with ranges between 77.2–93.2 % and 94.7–97.2 %, respectively. On the contrary, the plants of *T. rosea* and *C. odorata* showed a survival rate significantly lower in the treatments with petroleum in comparison with the control after 64 and 40-days of experimentation, respectively ($P<0.05$, Fig. 4). For *T. rosea* the final ranges of survival were between 28.8 % (C3) and 93 % (C0). The survival for C1 (81.9 %) was significantly greater ($P<0.05$) than C2 (50.5 %) and C3 ($P<0.05$). The differences were also significant between treatment C0 and the treatment with petroleum 40 days after the start of the experiment. *C. odorata* showed survival rates between 46.3 % (C3) and 86.5 % (C0). The differences between treatments were significant ($P<0.05$).

3.3 Tolerance Index

All of the species evaluated showed some degree of affect due to the presence of TPH in the soil. *S. macrophylla* showed the lowest TI with significant differences between the soil with petroleum and the control ($P<0.05$). *C. odorata*, *H. campechianum* and *T. rosea* were species with greater TI values (more affected from the treatments of petroleum in soil). The values of TI for these species in soil with petroleum were significantly different ($P<0.05$) in comparison with the C0 treatment. For all of the species, the differences between C1, C2 and C3 were not significant ($P<0.05$, Fig. 5).

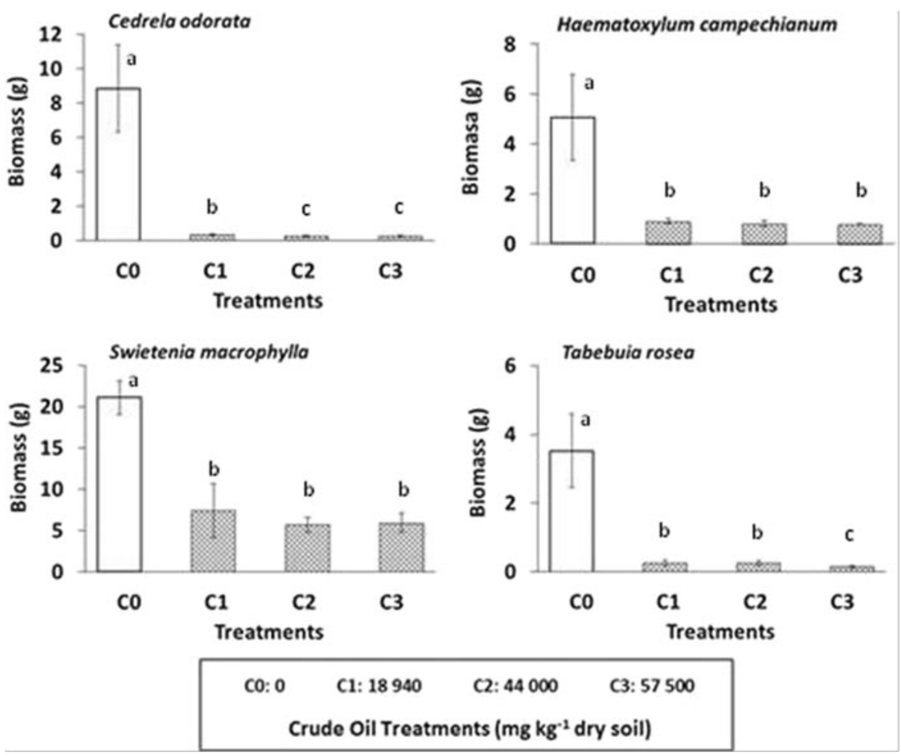


Fig. 3 Total mean dry biomass (aerial and root) of four tree species germinated on soil with four different treatments of heavy petroleum. For each specie, the treatments of petroleum with the same letter indicate that there are no significant differences ($P=0.05$)

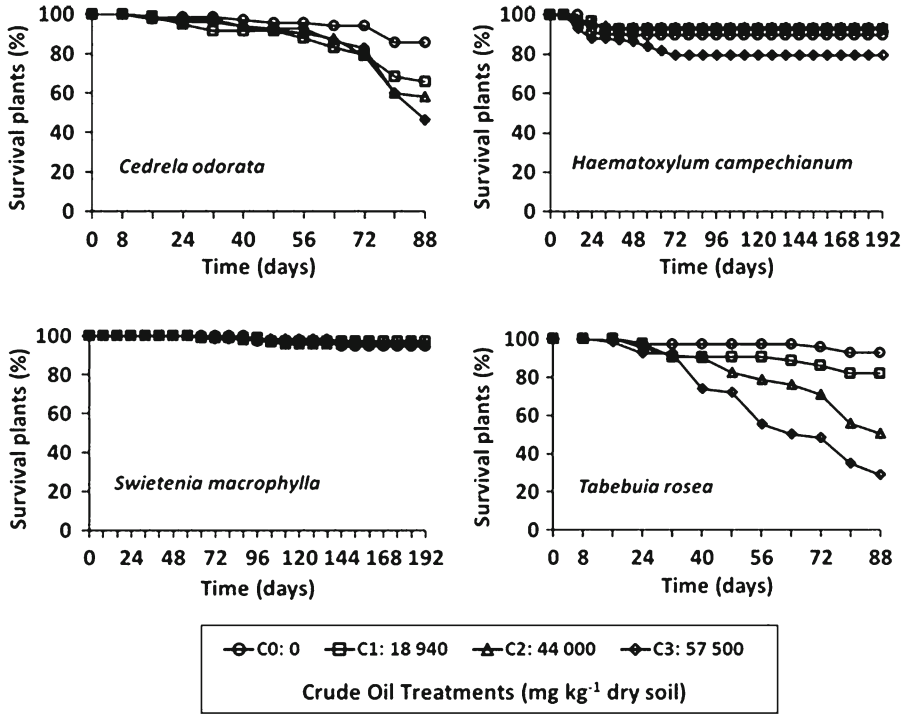
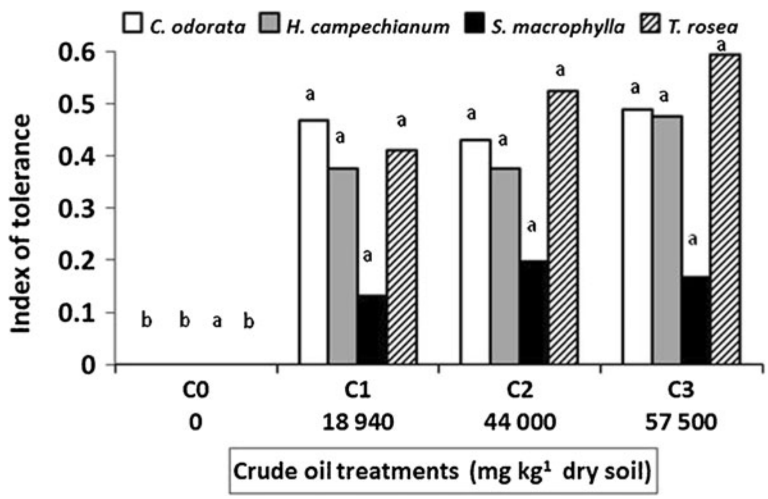


Fig. 4 Mean survival percent of four species of trees germinated on soil with four treatments of heavy petroleum

Although no significant differences were found between soils to which petroleum had been added, there were clear tendencies in the effects of petroleum in the soil on the various species. In Fig. 6 it can be seen that *T. rosea*, *C. odorata*, and *H. campechianum* showed values of TI very similar for the treatment C1. The TI for *H. campechianum* and *C. odorata* in the C2 treatment remains constant with a minimum variation with respect to C1. Meanwhile, in *T. rosea*, an exponential increase in TI was observed corresponding to the increase in petroleum concentration in soil. These three species showed an increase in the TI value in the C3 treatment. *S. macrophylla* showed very similar values between the three petroleum concentrations.

Fig. 5 Tolerance index of four species of trees germinated on soil with four treatments of heavy petroleum. Index=0, the petroleum does not cause an affect. Index <0, the petroleum stimulates the plant. Index >0, the petroleum negatively affects the plant. For each species, the treatment with the same letter indicates that there are no significant differences (*P*=0.05)



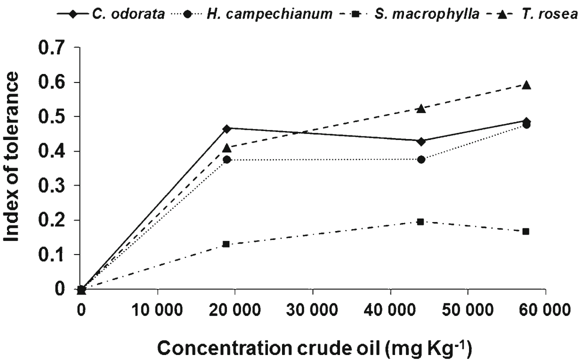


Fig. 6 Tolerance index of four species of trees germinated on soil with four treatments of heavy petroleum

4 Discussion

4.1 Germination

It is well known that there are differences in tolerance between vegetable species with respect to the toxicity of crude petroleum and its derivatives (Chaineau et al. 1997; Adam and Duncan 2002; Rivera-Cruz and Trujillo 2004; Besalatpour et al. 2008; Reynoso-Cuevas et al. 2008). The tolerance to petroleum hydrocarbons can vary as a function of species, and the type and concentration of petroleum and derivatives in the soil.

The arboreal species selected for this experiment are commonly used in reforestation programs in tropical areas of Mexico and Central America (Arriaga et al. 1994; Vázquez-Yanes et al. 1999; Cordero and Boshier 2003). *H. campechianum* is used less in reforestation, but due to its great utility as word for fence posts, firewood, live fences, and its use as a soil restorer (Cordero and Boshier 2003), it was also included. The seeds of these species do not show a latent period (Daws et al. 2005; Sautu et al. 2007), however it is recommended that they be soaked in water to promote and homogenize germination (Vázquez-Yanes et al. 1999; Gómez et al. 2006; CONAFOR 2013). When recently collected seeds are planted a high percentage of germination is achieved (Mostacedo and Fredericksen 2001). These species support periods of 6–12 months of storage, but the percentage of germination diminishes and the time for initiation and finishing germination increases according to the length of seed storage (Vázquez-Yanes et al. 1999; Vozzo 2002; Gómez et al. 2006). The germination percentages found in the treatments applied in this research are within the average times reported in other studies, where a planting substrate without petroleum was used in greenhouse conditions (Arriaga et al. 1994; Zamora-Cornelio et al. 2010; Chan-Quijano et al. 2012), with the exception of the control soil with *C. odorata* (57.5 %) and *S. macrophylla* (47.5 %).

Our results showed that seed germination of the species evaluated did not suffer any negative affect due to the presence of the heavy crude petroleum at the treatments used; on the contrary, the presence of petroleum in the soil increased and accelerated the seed germination of *S. macrophylla* and *C. odorata* up to 40 to 83 % and 60 to 80 %, respectively. The seeds of *T. rosea* also showed an acceleration of germination in the soil with petroleum during the first days of the experiment. In this respect, Adam and Duncan (2002) mention that some vegetable species have qualities that increase the germination rate in soils with petroleum hydrocarbons, and that a "factor" exists which causes the petroleum to stimulate the germination of certain species. Rivera-Cruz and Trujillo (2004) point out that the increase in germination due to the presence of petroleum appears to be linked with an increase in the entrance of water into the endosperm and the seminal cover of the seed, such that the enzymatic changes at the begging of germination occur in less time. Bossert and Bartha (1984) and Salanitro et al. (1997), mention that some petroleum compounds can function as growth promoter hormones. The increase in germination of *S. macrophylla*, *C. odorata* and the stimulation of *T. rosea* could be due to the fact that the seeds of these species, as fresh seeds, have a permeable seed coat which probably lets some petroleum hydrocarbons penetrate but which are not toxic at this phase of germination. On the contrary, they act as growth-promoting hormones, increasing and accelerating germination. This phenomenon was not observed in the germination of *H. campechianum* maybe because the seeds of this species were planted together with the fruit, which could have formed a physical barrier limiting the penetration of the petroleum hydrocarbons. Our results coincide with Adam and Duncan (2002) for *Agrostis stolonifera*, *Festuca rubra* and *Medicago sativa* which were found to have an increase in germination rate between 7 and 27 % in soil with 25,000 mg kg^{-1} of diesel. Sharifi et al. (2007) observed an increase in germination of 84 % for *Triticum sativa* and 43 % for *Medicago truncatular* in 25,000 mg kg^{-1} of motor oil and with 50,000 mg kg^{-1}, the germination rates were increased 34 and 19 %, respectively. This is in agreement with Rivera-Cruz and Trujillo (2004) for *Brachiaria mutica* and *Mimosa pigra* in which an increase of 13 and 117 % of germination was observed in soil with 79,457 mg kg^{-1} of weathered petroleum. These authors also observed an increase of 117 and 185 % in the germination of *Cyperus articulatus* and *Cyperus* sp., respectively, at a concentration of 9,035 mg kg^{-1}.

The fact that no negative effect was encountered in the germination of seeds in the presence of petroleum could be explained due to the type of petroleum used in this study (heavy crude), which has a low content of the light hydrocarbon fraction (composed of five to ten carbons, SEMARNAT 2005). Also, having let the recently contaminated soil set for 5 days at ambient conditions in a tropical environment before planting could have caused the volatilization of the lighter fraction and possible part of the medium fraction (ten to 28 carbons, SEMARNAT 2005). In general, the lighter fraction hydrocarbons are more toxic than hydrocarbons in the medium and heavy fractions (Chaineau et al. 1997; Adam and Duncan 2002; Vázquez-Luna et al. 2010). Hydrocarbons from the light fraction are characterized as been more volatile, and can easily move through the cell membranes, penetrate, and harm the seed embryo, thus resulting in the reduction in seed germination (Labud et al. 2007; Reynoso-Cuevas et al. 2008). Adam and Duncan (2002) found that the germination of 25 different species including grasses, herbs, legumes, and commercial crops increased significantly when the mobility of the lighter fraction of diesel was inhibited, at soil concentrations of 25,000, and 50,000 mg kg^{-1}. On the other hand, petroleum hydrocarbons in soil tend to form a hydrophobic layer that covers the seed, interrupting the interchange of gases and air (Li et al. 1997; Sawatsky and Li 1997). This physical barrier has been pointed to be as the principal cause in the delay of seed germination when the concentration and type of petroleum are not toxic to the vegetable species (Chaineau et al. 1997; Adam and Duncan 2002). In this study this phenomenon seems not to have had the same effect since the necessary humidity was maintained in the soil (in greenhouse conditions), sufficient to moisten this layer and allow seed germination. In this sense Li et al. (1997) point out that when the soil is maintained near field capacity, soil water repellence can be almost undetectable. Also, the pre-germination treatment applied to the seeds allowed them to imbibe sufficient water in the cotyledons to initiate germination and develop.

4.2 Plant Development

During the first days after seed germination no differences were observed in the development of the plantlets (height, biomass and survival) between the soil with petroleum and the control. This is attributed to the large energy reserve in the cotyledons of the species evaluated, which provided sufficient energy to be able to complete the germination process without a visible effect due to the concentration of petroleum in the soil. Vázquez-Luna et al. (2010) argued that the cotyledons of *Leucaena leucocephala* had a greater quantity of reserves that allowed the plantlet to maintain itself, independently of its environment during a longer time period. Maybe for this reason, we observed the same size, coloration and vigour in the plantlets of the species evaluated during the first weeks after germination. In this respect, Hampton and Coolbear (1990) mentioned that the differences in the vigour of the plantlet is associated principally with the quantity of reserves and the metabolic efficiency, which could represent an advantageous condition for species with cotyledons of greater size, such as is the case of the species evaluated in the present study. However, after 2 weeks differences began to be observed in the effects of petroleum on each species. The reduction in biomass due to the effect of petroleum was notable for all species studied. Only *S. macrophylla* did not suffer any significant effect on plant height, and along with *H. campechianum*, did not suffer any effect on survival. The degree of effect on plant development, as with germination, depends on the type of petroleum or derivatives, concentration, and exposure time, and especially the plant species (Adam and Duncan 2002; Rivera-Cruz and Trujillo 2004; Brandt et al. 2006). In this respect, Brandt et al. (2006) found a reduction of 50 % in the total biomass and of 40 % in plant height in the grass *Vetiveria zizaniodes* in soil with 50,000 mg kg^{-1} of crude petroleum during a 6-month experiment. Rivera-Cruz and Trujillo (2004) reported a reduction on the biomass in soil with fresh petroleum for *B. mutica*, *C. articulatus*, *Cyperus* sp. and an increase in biomass for *M. pigra* and *Echinochloa polystachya* in concentrations up to 100,000 mg kg^{-1} of fresh petroleum. Salanitro et al. (1997) reported an increase in biomass of 40 and 70 % of *Zea mays* in soil contaminated with medium crude petroleum (30° API) at 26,600 mg kg^{-1} and heavy crude (14° API) at 14,000 mg kg^{-1}, respectively. For the same concentrations and types of petroleum the same authors also report a decrease in biomass for *Triticum aestivum* and *Avena sativa*.

The decrease in biomass, height and survival of the plantlets of the species used in this study can be attributed to the presence of petroleum in the soil, and even though it was no very toxic to the species studied, it did affect soil fertility. The medium range hydrocarbons and above all, the heavy range hydrocarbons tend to cover the surfaces of soil particles, interrupting the electrostatic interaction with some nutrients of importance to plants (NH_4^+, K^+, Ca^{++}), reducing their availability (Roy and McGill 1998). On the other hand, medium-range and heavy-range hydrocarbons have low polarity and are highly attracted to plant roots (Briggs et al. 1982; Burken and Schnoor 1998). These hydrocarbons create a hydrophobic layer around the root which limits the absorption of nutrients and the interchange of gases. Under these conditions, the plants suffer a metabolic imbalance generated by a condition of oxidative stress which disrupts cellular homeostasis (Mittler 2002; Gill and Tuteja 2010).

Oxidative stress is the toxic effect caused by chemical substances that are highly reactive (reactive oxygen species, ROS) produced during the reduction of molecular oxygen (Halliwell 2006). Upon entering into a stressed state the plants act to reduce the production of ROS by processes such as anatomical and physiological adaptations, mechanisms which reorganize the photosynthetic apparatus, or suppress photosynthesis (Gechev et al. 2006). Another form of defence is the action of antioxidant molecules to eliminate ROS. If the intercellular concentration of ROS is not controlled, a direct consequence is the damage of cell structure, as well as the interruption of metabolic pathways (Blokhina et al. 2003; Moller et al. 2007); conditions which can cause plant death. Possibly, it is by this phenomenon that *C. odorata* and *T. rosea* were seen to be drastically affected in the production of biomass, height and especially, plant mortality. For these two species the mortality was very high at 88 days, due to which the plants were harvested before the conclusion of the experimental period. Upon harvesting the plants it was observed that their roots were completely covered in a layer of petroleum, being more notable in the soil with the highest concentration. Thus, it appears that *S. macrophylla*, and to a lesser degree *H. campechianum*, possess a better antioxidant defence that acts of protect the cells from possible oxidative damage (Moller 2001; Gratáo et al. 2005). These conferred them the capacity to survive in the soil with petroleum, with effects only to height and biomass. In relation to this Martí et al. (2009) found a correlation between the increase in antioxidant enzyme activity and plant tolerance to petroleum muds in *M. sativa*. For *Melilotus albus* developing in the presence of diesel, an over production of ROS was registered that stimulated the activity of antioxidant enzymes (Hernández-Ortega et al. 2011). On the other hand, microorganisms also play an important role in the reduction of oxidative damage caused by the presence of TPH. Debiane et al. (2009) found that the fungus *Glomus intraradices* notably reduced the oxidative damage in *Chichorium intybes* caused by the presence of anthracene and benzo(a)pyrene. On the other hand, Guerrero-Zúñiga and Rodríguez-Dorantes (2009) found that plants of *Cyperus hermaphroditus* responded to the toxicity of phenanthrene with a greater enzyme activity and expression of proteins in the rhizospheric zone, and that these exudates can transform or participate in the partial transformation of toxic products to less toxic substances which may be more available to the roots or the rhizospheric microorganisms (Gianfreda et al. 2005). Root exudates (sugars, alcohols, acids and enzymes) can also provide sufficient carbon and energy for rhizospheric microorganisms (Schnoor et al. 1995). Maybe, as suggested by Walton et al. (1994), *S. macrophylla*, and to a lesser extent *H. campechianum*, upon going into stress due to the presence of petroleum in the soil, responded to the increase by a change in rhizospheric exudates, which then caused modification in the rhizospheric microflora in composition or activity, resulting in the degradation of the contaminants in the rhizosphere, thus reducing the layer of petroleum surrounding the roots of these species. It was observed that there was less impact to these two species, being able to survive in the presence of petroleum in the soil.

4.3 Tolerance Index

The tolerance index integrates and weights the effects caused in the germination and development of plants in soil with petroleum or its derivatives. This index provides a numeric value of all of the variables evaluated. This value can serve to determine, in a holistic way, all of the behaviour of a species in hydrocarbon contaminated soil. A similar value of TI was shown for *C. odorata* and *H. campechianum* at the C1 and C2 concentrations indicating that, although they are significantly affected by the petroleum, their behaviour is the same at both concentrations. However, at the C3 concentration, it appears that a threshold is surpassed for these species. These two species, together with *T. rosea*, very probably can develop well at petroleum concentrations less than 20,000 mg kg^{-1}. In future experiments it will be important to test lower petroleum concentrations (maybe 3,000, 6,000, 9,000, 12,000, 15,000 and 18,000 mg kg^{-1}) to be able to define a threshold for these species, and thus be able to select an appropriate species based on the concentration of petroleum in the soil where phytoremediation is being considered.

In the present investigation the result of TI for *S. macrophylla* indicates that it is a species with qualities that allow it to develop in soil with heavy crude petroleum at concentrations up to 57,000 mg kg^{-1} TPH. It appears that this species has metabolic mechanisms that activate upon entering into a stressed condition due to the presence of petroleum in the soil. With these results continued research is indicated to determine the mechanism by which this species tolerates heavy crude petroleum, and also to determine its long-term establishment and development in contaminated soil.

5 Conclusions

The response to heavy crude petroleum-contaminated soil was different for each vegetable species studied. Seed germination was not negatively affected due to the presence of heavy crude. The germination of *C. odorata* and *S. macrophylla* increased and was stimulated in the presence of fresh heavy crude petroleum. The height and biomass of all the species evaluated decreased in soil with heavy crude oil. The effect of petroleum in the survival of plantlets of *C. odorata* and *T. rosea* was drastic, in which a tendency of less survival in petroleum-contaminated soil was observed. The plantlets of *S. macrophylla* and *H. campechianum* had an equal survival rate in soil with or without petroleum; however, they suffered significant effects with respect to biomass accumulation and plant height. *S. macrophylla* turned out to be the most tolerant species at the concentrations, and petroleum type tested. This species could be propagated in hydrocarbon-contaminated soils. However, it is necessary to test this in the field to determine its behaviour under natural conditions. The sensibility of the plants to heavy crude petroleum, according to the TI value, increases with increasing concentration of petroleum in soil. It is recommended to run phytoremediation tests with *S. macrophylla*, since this specie is tolerant of heavy crude petroleum, and thus it is necessary to determine its capacity with respect to the degradation of petroleum hydrocarbons in soil. The use of tree species could be important because of the great potential of trees for phytoremediation due to their long life, biomass and deep roots that can penetrate and remediate deeper soil layers.

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