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# Physiological responses of *Quercus oleoides* (Schltdl & Cham) to soils contaminated by diesel

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**ABSTRACT**

Oil pollution is a worldwide threat to the environment that affects the development of plants. The effect of soil contaminated by diesel on the physiological responses of seedlings of *Quercus oleoides* was investigated in two independent experiments. We proposed that physiological performance will decrease when seedlings are exposed to higher concentration of contamination. At the first experiment, levels of pollution with diesel were of 0%, 5%, 10% and 15%, and 0%, 2% and 3.5% in the second one. In the first experiment, photosynthetic rate, stomatal conductance, transpiration and total chlorophyll of *Q. oleoides* were higher in the control seedling, and lower in treatments of pollution with 5%, 10% and 15% of diesel during 3 days of treatment. Only, seedlings in soil polluted with 5% of diesel survived up to 16 days; they showed a photosynthetic rate of 5 μmol $m^{-2}$ $s^{-1}$, which was lower than control seedlings (9 μmol $m^{-2}$ $s^{-1}$). This pattern was observed in stomatal conductance, transpiration and relative water content. Surprisingly, in the second experiment, seedlings showed a higher photosynthetic rate and growth at 2% of diesel-contaminated soil than control seedlings, a phenomenon known as hormesis. In both experiments, soil respiration was proportional to soils contaminated. We concluded that *Q. oleoides* is highly vulnerable in soils contaminated with above 5% of diesel, but it maintains its physiological activities in soils contaminated below 2%, suggesting that seedlings can grow under low concentration of diesel contaminant, and may be used in phytoremediation of soils with low concentrations of diesel contamination.

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Diesel; phytoremediation; photosynthesis; hormesis; *Quercus oleoides*

## 1. Introduction

Oil pollution is a worldwide threat (Li et al., 1997; Peña-Castro et al., 2006; Pérez-Hernández et al., 2013). Some activities related to oil extraction, transportation and processing, in many instances, contaminate both soil and water, which affect all species including plants, animals and microorganisms (García et al., 2004; Iturbe et al., 2005). Soil pollution by hydrocarbons forms thick layers on the surface affecting the porosity and reducing air spaces. Once formed, this layer acts as water repellent, i.e. highly hydrophobic reducing water hold infiltration and retention causing water stress on plants (Racine, 1994). Hydrocarbons in soil induce organic matter accumulation and increase soil acidity; this affects bases saturation and cationic exchange capacity from soil to plants (Adam & Duncan, 2002).

Hydrocarbons have negative effects on plant tissues, inducing necrosis of foliage, senescence and defoliation, followed by general impaired growth and plant mortality (Adam & Duncan, 2002; Zhang & Kirkhan, 1996). At the structural level, hydrocarbons have a negative influence on the development of the plant root system, and this may induce a reduction in water transportation and transpiration (Zhang & Kirkhan, 1996). Furthermore, photosynthesis rate also be reduced when plants are exposed to diesel, for example, plants experiment reduce in $CO_2$ uptake (Eberhard & Wollman, 2008).

Soil contaminant reduction can be carried out by different techniques such as excavation, burning or soil wash, but all are expensive. In counterpart, the use of plants can be a non-expensive alternative (Delgadillo-López et al., 2011), which is environmentally friendly when soils are contaminated by hydrocarbons. This process is known as phytoremediation.

Some plant species can help to clean and stabilize a soil contaminant if its concentration is not phytotoxic (Cunningham et al., 1996). Several studies have demonstrated that degradation of hydrocarbons and some of their components (e.g. aliphatic or polycyclic hydrocarbons, phenols and others) may occur faster in soils covered with vegetation than those with microorganisms or sterile (Muratova et al., 2003;

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Rivera-Cruz et al., 2005; Siciliano et al., 2003). Plants are considered to have an active role in transformation hydrocarbons fractions, similar to that occurring in the rhizosphere, where soil microbial diversity is increased and it propitiates a higher degradation activity on contaminants due to the multiple enzymatic activities carried out by microorganisms (Mishra et al., 2001; Muratova et al., 2003; Rivera-Cruz et al., 2005). In fact, plants affected by contaminants may change their exudation patterns in order to promote growth of microorganisms that are able to degrade contaminants (Siciliano & Germida, 1998).

Hydrocarbons are one of the main soil and water contaminants as a result of the activities of the oil industry with negative effects on ecosystem functioning, because it affects its structure and bioprocesses.

Among the pollutants in Mexican soils, diesel was the most contributed after gasoline, with14.1% of the environmental emergencies reported by PROFEPA (Procuraduría Federal de Protección al Ambiente) between 2008 and 2015 (PROFEPA, 2016). Particularly, this contaminant has been detected in concentrations of up to 5.272 mg/kg with average values of 437 ± 1.277 mg/kg in soils of southwestern in Mexico (Iturbe et al., 2006), which represents a risk to human health. This situation occurs in the communities surrounding the zone of oil companies in the southern of Tamaulipas state, Mexico, where tropical oak forests, i.e. *Quercus oleoides*, are distributed forming disperse communities close to the continental littoral (Pennington & Sarukhán, 2005). The use of tree species in the phytoremediation of soils contaminated by organic substances is still very limited, being *Pinus sylvestris, P. deltoides, Picea abies* and *Casuarina equisetifolia* the most used for these purposes (Palmroth et al., 2002; Schoenmuth & Pestemer, 2004; Sun et al., 2004), while herbaceous species such as *Lolium perenne* and *Trifolium repens* have been extensively used (Kaimi et al., 2006; Palmroth et al., 2002; Tang et al., 2010). The ability of plants to establish and grow on hydrocarbons polluted soils is one of the characteristics sought after in order to determine their use in phytoremediation. The most of publications report the dynamics of microorganisms for growth on polluted soils (Castro-Mancilla et al., 2013; Labud et al., 2007; Méndez-Cabrera et al., 2005; Salazar-Sosa et al., 2003), but few have focused in the physiological responses of plants to diesel-contaminated soils. In this context, we address the question, how does diesel-polluted soil affect the gas exchange, water relations and growth of *Q. oleoides* seedlings under greenhouse conditions? We expected that gas exchange, water relations and growth will decrease when *Q. oleoides* are exposed to higher concentration of diesel in the soil.

## 2. Materials and methods

### 2.1. Plant material

One-year old seedlings of *Q. oleoides* obtained from the greenhouse at the 'Instituto Potosino de Investigación Científica y Tecnológica de San Luis Potosí' were used. These seedlings were obtained from seeds collected at the community El Salto (22°27′48.7″ N and 100°42′5.2″ W), in the north of San Luis Potosí, Mexico. Seedlings were transported to the greenhouse at the 'Instituto de Ecología Aplicada' from the 'Universidad Autónoma de Tamaulipas', where they were acclimated for 20 days in a common garden at a temperature of 28 ± 4°C and relative humidity of 71 ± 14%. Soil obtained from the municipality of Ocampo, Tamaulipas, which is similar to soil from El Salto was used. Soil was extracted from the first 20 cm depth from the surface, air dried and sieved (2 mm); for its characterization, see Table 1.

Tolerance of *Q. oleoides* seedlings to diesel-polluted soil was investigated in two independent experiments. In the first, contamination levels were of 5%, 10% and 15% weight/weight considering as reference the studies reported by Castro-Mancilla et al. (2013) and Maldonado (2013). The second one was carried out after the first experiment, here minimal tolerance levels of *Q. oleoides* seedlings were studied (0%, 2% and 3.5% weight/weight). Prior to the experiment, plants were watered in order to keep 40% humidity level, after that, plants were kept watering until to reach field capacity. We have four replicates for each polluted soil and non-polluted soil.

**Table 1.** Physical–chemical characterization of soil used in experiments I and II.

| Parameter | Result | Analytical method |
|---|---|---|
| Texture | Franc | Bouyucos (Fernández et al., 2006). |
| Clay | 24.70% | |
| Silt | 33.24% | |
| Sand | 42.40% | |
| pH | 7.25 | Potentiometer in relation 2:1 (water–soil). |
| Electric conductivity | 114 mS $cm^{-1}$ | Conductimeter in soil extract (Fernández et al., 2006). |
| Organic material | 8.57% | Walkley and Black (Galantini, Rossel & Iglesias, 1994). |
| Organic carbon | 4.97% | Walkley and Black (Galantini et al., 1994). |
| Total nitrogen | 0.49% | Walkley and Black (Galantini et al., 1994). |
| Extractable phosphorus | 0.25 mg $kg^{-1}$ | Olsen (Muñoz, Mendoza, López, Soler & Hernández, 2003). |
| Exchangeable potassium | 5.1 meq $100^{-1}g$ | Bray y Kurtz No. 1 modified (Fernández et al., 2006). |
| Apparent density | 1.83 g $cm^3$ | AS 03 (NOM 021 SEMARNAT). |
| Field capacity | 30% | Fernández et al., 2006. |

SEMARNAT: Secretaria de Medio Ambiente y Recursos Naturales.

### 2.2. Experiment I

Soil samples were polluted with 5%, 10% and 15% weight/weight diesel and non-polluted soil was used as control. Plastic bags with 4 kg capacity were used for the experiments, soil was mixed and the corresponding treatment was applied. The experiment lasted for 16 days and the following variables were measured.

### 2.3. Physiological traits

Transpiration ($E$, mmol $H_2O$ $m^{-2}$ $s^{-1}$), stomatal conductance ($g_s$, mmol $H_2O$ $m^{-2}$ $s^{-1}$) and photosynthetic rate ($A$, µmol $CO_2$ $m^{-2}$ $s^{-1}$) were measured on healthy, fully expanded leaves of *Q. oleoides* using a portable gas exchange system LI-6400 (LI-COR, Inc., Lincoln, NE, USA).

For this procedure, the second leaf from each seedling was used. $CO_2$ to 400 ppm values were calibrated on the portable system using $CO_2$ cartridges (cylinders LI-COR of 12 g). Light was adjusted using the LI-6400 (red + blue light source) as a light source, modifying intensity to 200, 300, 600, 800, 1000, 1200 and 1800 µmol $m^{-2}$ $s^{-1}$ of photosynthetic photon flux density (PPFD). Light was applied in a decreasing order, with 2 min intervals between each reading. Use of decreasing light rather than increasing light reduces the equilibrium time required for stomatal opening and photosynthetic induction (Kubiske & Pregitzer, 1996). The relative humidity was of 50%. Leaf temperature during measurements was maintained at 34.2 ± 1.9°C in the first experiment and 28.6 ± 0.45°C in the second one. Mean vapor pressure deficit (VPD) at the different irradiances ranged from 1.4 to 2.7 kPa for the first experiment and 1.2 to 1.9 kPa for the second one, with a flow rate of 400 µmol $s^{-1}$ in both experiments. Measurements were made between 9:00 and 11:00 h at 3, 9 and 16 days from the beginning of treatment.

Total chlorophyll was measured using the Minolta® SPAD 502 chlorophyll meter measuring transmitted light through the leaf by two bands differing in optical density: 650 and 940 nm. Measurements were made on healthy leaves for seedlings in the morning, around 10:00 h at 3, 9, 11 and 16 days from the beginning of the treatment. The leaf used for gas exchange variables was also used for chlorophyll measurements.

Relative water content (RWC) was measured in a piece of healthy leaf sample (1 $cm^2$) at predawn. Samples were placed in a plastic bag with moist filter paper, placed within an insulated box with ice, transported to the laboratory to measure their fresh weight, placed in distilled water to obtain saturated weight, and then dried at 65°C during 24 h before measuring dry weight. The RWC was calculated as (fresh weight − dry weight)/(saturated weight − dry weight) × 100. Measurements were taken at 3 and 16 days from the beginning of the treatment.

### 2.4. Experiment II

Because most seedlings of *Q. oleoides* died at 11 days from the beginning of the experiment I, we established the second experiment, where diesel pollution levels below 5% were evaluated. Pollution treatments used were 2% and 3.5% weight/weight diesel, and a non-polluted soil was used as control. They were set up pretending to surpass the maximum allowed levels established by the Mexican Official Norm (NOM-138-2012, Norma Oficial Mexicana, 2012). Physiological variables as in experiment I were evaluated, and measurements were made at 15, 30 and 50 days from the beginning of the experiment.

### 2.5. Soil respiration

We have soil with and without seedlings of *Q. oleoides* of each treatment of diesel pollution to evaluate $CO_2$ production using a PBI (Dansensor Check Mate II, Spain). Diesel-polluted soil samples (30 g) for each treatment with and without seedling at 28°C for 24 h were used (García et al., 2003) and $CO_2$ production was determined. Measurements were made in four replicates of each diesel-polluted soil level at 15 days for experiment I and at 50 days for experiment II.

Plant height and number of leaves were measured at the beginning and end of experiment II, with four replicates per treatment were taken.

### 2.6. Data analysis

Photosynthesis rate, stomatal conductance and transpiration of seedlings were compared using a one-way analysis of variance (ANOVA), among pollution levels (0%, 5%, 10% and 15% for experiment I and 0%, 2% and 3.5% for experiment II). PPFD values were non-significant within treatment; therefore, we considered one mean for each treatment to following comparisons. A one-way ANOVA was also used to compare total chlorophyll, RWC and seedlings growth among soil pollution levels in each experiment. Homogeneity of variances and normality was checked before to use ANOVA, and continuous data were log transformed. We used a Kruskal–Wallis test when variables were not normally distributed. A two-way ANOVA was used to evaluate the soil respiration in experiments I and II, considering the presence of seedlings in pots and the soil pollution levels as factors. Values of $P \leq 0.05$ were accepted as significant. Statistical tests were performed with STATISTICA 9.0 (StatSoft Inc., Tulsa, OK, USA).

## 3. Results

### 3.1. Experiment I

Photosynthesis rate (*A*), stomatal conductance ($g_s$) and transpiration (*E*) of seedlings of *Q. oleoides* were negatively affected by diesel pollution at 3, 9 and 16 days of treatment (Figure 1). Low values of these physiological variables were directly related to soil pollution levels.

After 3 days, control seedlings showed higher photosynthesis rate (*A*) compared to seedlings growing under 5%, 10% and 15% of soil polluted at 300, 600, 1000 and 1200 µmol $m^{-2}$ $s^{-1}$ of PPFD ($F_{3,\ 12} = 6.83$, $P < 0.05$ for all levels of PPFD; Figure 1(a)). Control seedlings had an *A* of 8.55 ± 0.79 µmol $CO_2$ $m^{-2}$ $s^{-1}$ at 1200 µmol $m^{-2}$ $s^{-1}$ of PPFD, while in polluted seedlings (5%, 10% and 15%) it was lower than 6.40 µmol $CO_2$ $m^{-2}$ $s^{-1}$.

Also, control seedlings had higher values of $g_s$ and *E* than seedlings in soil polluted (Figure 1(b,c)). Thus, control seedlings had a $g_s$ of 78 ± 7.24 mmol $m^{-2}$ $s^{-1}$, and seedlings in soil polluted had values lower than 20 mmol $m^{-2}$ $s^{-1}$ ($H = 53.24$, $df = 3$, $P < 0.01$; Figure 1(b)). Moreover, control seedlings had an *E* of 0.98 ± 0.42 mmol $m^{-2}$ $s^{-1}$, which decreased by 60% under the 5%, 10% and 15% polluted soil ($H = 55.19$, $df = 3$, $P < 0.01$; Figure 1(c)).

After 9 days from establishment, seedlings under 15% soil pollution showed wilting leaves and measurements were stopped. We observed a clear difference in *A*, $g_s$ and *E* between control seedlings and those in the 5% and 10% treatments. Control seedlings showed an *A* maximum of 10.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$ at 300 µmol $m^{-2}$ $s^{-1}$ of PPFD, which was significantly higher than those in the 5% and 10% treatments ($F_{2,\ 9} = 6.28$, $P < 0.05$; Figure 1(d)). The maximum values for $g_s$ were 88 ± 2.92 mmol $m^{-2}$ $s^{-1}$ in control seedlings, and it was 80% lower in seedlings in the 5% treatment; and values were close to 0 in those under the 10% treatment ($F_{2,\ 9} = 55.1$, $P < 0.05$; Figure 1(e)). Control seedlings showed *E* values threefold higher than those under the 5% and 10% treatments ($F_{2,\ 9} = 59.45$, $P < 0.05$; Figure 1(f)).

After 16 days, only control seedlings and those under the 5% treatment survived; the first showed an *A* maximum value of 7.9 ± 0.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$, which diminished by 50% in the second (Figure 1(g); $F_{1,\ 6} = 9.65$, $P < 0.05$). The $g_s$ and *E* followed the same pattern than *A*, i.e. control seedlings showed higher values than those under the 5% treatment ($F_{1,\ 6} = 17.73$, $P < 0.05$; Figure 1(h); $F_{1,\ 6} = 21.77$, $P < 0.05$; Figure 1(i)).

Total chlorophyll did not show significant differences at 3 ($F_{3,\ 12} = 1.26$, $P > 0.05$), 9 ($F_{2,\ 9} = 0.51$, $P > 0.05$) and 16 days ($F_{1,\ 6} = 2.89$, $P > 0.05$; Figure 2) among treatments. Moreover, this variable did not show a significant variation for each of the pollution treatments during the experiment ($P > 0.05$).

The RWC of control seedlings was 89 ± 25.4% and was reduced in a 30% in seedlings under the 5%, 10% and 15% treatments after 3 days; although these values were only

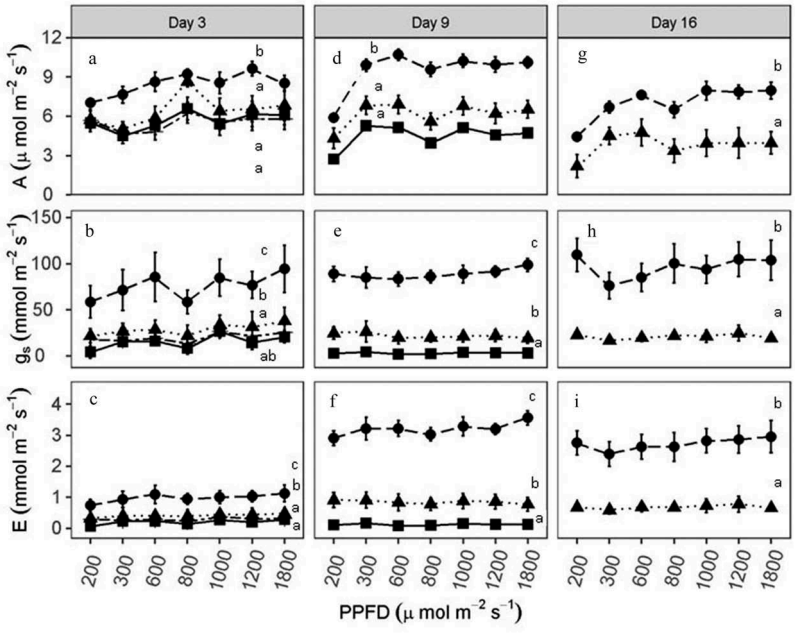


Figure 1. Rhythm of the photosynthetic rate (A), stomatal conductance ($g_s$) and transpiration (*E*) of *Quercus oleoides* seedlings in response to different photosynthetic photon flux densities (PPFD) at 3, 9 and 16 days of soil diesel pollution. Different lowercase letters denote significant differences among diesel treatment within each panel ($P < 0.05$). Each point represents the mean ± SE ($n = 4$). Circle represents 0% treatment, triangle 5% treatment, square 10% treatment and diamond 15% treatment.

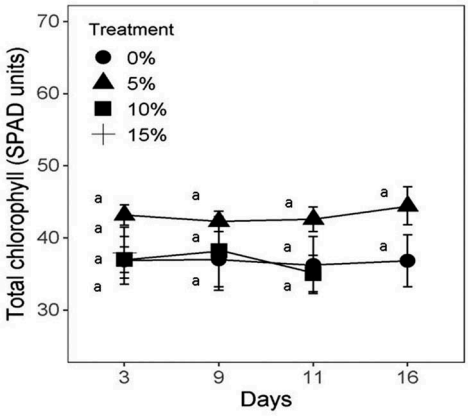


**Figure 2.** Total chlorophyll of *Quercus oleoides* under three levels of soil diesel pollution (5%, 10% and 15%) and in control seedlings. Different letters mean significant differences among diesel treatment on each day ($P < 0.05$). Each point represents the mean ± SE ($n = 4$). Note: only one measurement was made in the seedlings under the 15% of contamination because leaves were wilting after day 11 of treatment.

significant for seedlings in the 15% treatment ($F_{3, 12} = 3.59$, $P < 0.05$; Figure 3). After 16 days, the RWC was higher in control seedlings than those in the 15% treatment (73.5 ± 5 vs. 51.6 ± 5%; $F_{1, 6} = 9.62$, $P < 0.05$; Figure 3).

The interaction of seedlings presence and pollution levels affected soil respiration ($F_{2, 12} = 53.60$, $P < 0.05$; Table 2; Figure 4). Soil respiration was proportional to soil pollution; it was higher in the 5% treatment

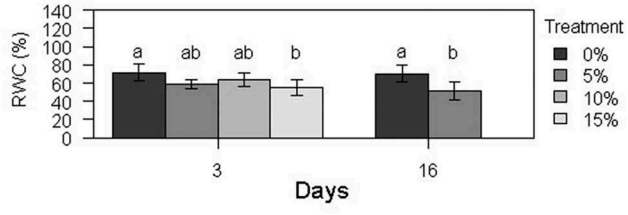


**Figure 3.** Relative water content (RWC) of *Quercus oleoides* leaves under three levels of soil diesel pollution (5%, 10% and 15%) and in control seedlings. The measurements were made at the beginning and end of the experiment. Different letters mean that the treatments are statistically different ($P < 0.05$). Each bar represents the mean ± SE ($n = 4$).

**Table 2.** Analysis of the two-way ANOVA to estimate the effect of the presence of seedlings and the percentage of contamination with diesel in edaphic respiration.

| Sources of variation | SS | *df* | MS | *F* | *p* |
|---|---|---|---|---|---|
| Presence of seedlings | 0.03556 | 1 | 0.03556 | 2.286 | 0.156 ns |
| % diesel polluted | 21.13000 | 2 | 10.5650 | 679.17 | **<0.05*** |
| Presence of seedlings × % diesel polluted | 1.66778 | 2 | 0.83389 | 53.60 | **<0.05*** |
| Error | 0.18667 | 12 | 0.01556 | | |

Each value represents the mean ± SE.
In Bold: *Significant differences among contamination treatments, $P < 0.05$.

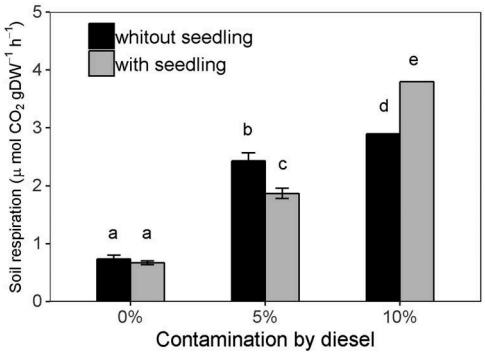


**Figure 4.** Soil respiration in treatments of 0%, 5% and 10% of soil diesel pollution. The measurement was performed at 18 days of treatment. Different letters mean that the treatments are statistically different ($P < 0.05$). Each point represents the mean ± SE ($n = 4$).

without seedlings than the one with seedlings. This result was opposite in the 10% treatment ($F_{2, 12} = 53.60$, $P < 0.05$; Figure 4).

### 3.2. Experiment II

After 15 days, seedlings showed a photosynthesis rate of 6.3 ± 0.9 μmol $CO_2$ $m^{-2}$ $s^{-1}$ in the 2% treatment, which was higher than control seedlings, even higher than seedlings in the 3.5% treatment ($F_{2, 9} = 4.24$, $P < 0.05$; Figure 5(a)). However, the $g_s$ and $E$ were 20% higher in the control seedlings than in those in the 2% ($F_{2, 9} = 4.37$, $P = 0.05$; Figure 5(b)) and 3.5% treatments ($F_{2, 9} = 4.40$, $P < 0.05$; Figure 5(c)).

After 30 days, there were no differences in photosynthesis rate between control seedlings and those in the 2% treatment (6.12 ± 0.02 vs. 6.63 ± 0.02 μmol $CO_2$ $m^{-2}$ $s^{-}1$; $F_{2, 9} = 5.64$, $P > 0.05$; Figure 5(d)). Particularly, photosynthesis rate was higher only in seedlings in the 2% than 3.5% of soil polluted ($F_{2, 9} = 5.64$, $P < 0.05$; Figure 5(d)). The $g_s$ and $E$ values of control seedlings were 50% higher in comparison to those in polluted soil ($F_{2, 9} = 11.47$, $P < 0.05$ in the 5% treatment, Figure 5(f); $F_{2, 9} = 10.11$, $P < 0.05$ in the 10% treatment; Figure 5(e)).

After 50 days, again, seedlings in the 2% treatment showed higher photosynthesis rates than those in the 3.5% treatment, and that of control seedlings ($F_{2, 9} = 4.19$, $P = 0.05$; Figure 5(g)). On the other hand, nonsignificant differences were found for $g_s$ and $E$ between control seedlings and those in the 2% treatment ($F_{2, 9} = 4.60$, $P > 0.05$; Figure 5(h); $F_{2, 9} < 5.12$, $P > 0.05$; Figure 5(i), respectively).

At 11 and 30 days, nonsignificant differences for total chlorophyll were found among treatments (0%, 2% and 3.5%) ($F_{2, 9} = 3.74$, $P > 0.05$; Figure 6). However, seedlings under the 2% treatment showed

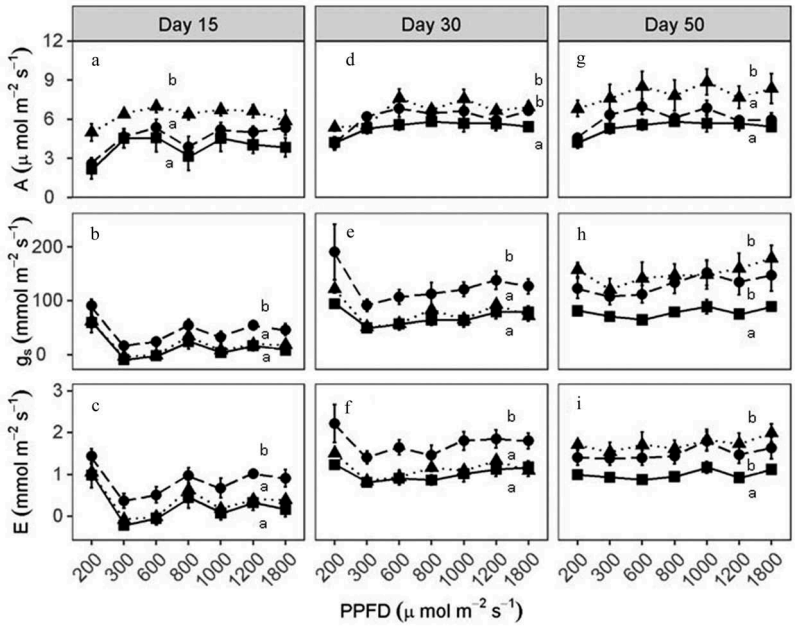


**Figure 5.** Photosynthetic rate (A), stomatal conductance ($g_s$) and transpiration ($E$) in leaves of *Quercus oleoides* in different photosynthetic photon flux densities (PPFD) at 15, 30 and 50 days of treatment. Different lowercase letters denote significant differences among diesel treatment within each panel ($P < 0.05$). Each point represents the mean ± SE ($n = 4$). Circle represents 0% treatment, triangle 2% treatment and square 3.5% treatment.

30% more total chlorophyll than those under the 3.5% and control seedlings at 15 and 50 days ($F_{2,\ 9} < 5.40$, $P < 0.05$; Figure 6).

At 50 days from the experiment, soil respiration was increased at the 2% and 3.5% soil polluted than control seedlings ($H = 7.73$, $df = 2$, $P < 0.05$; Figure 7).

Seedlings in the 2% treatment showed higher growth (20%) in comparison to control seedlings at 46 days ($F_{2,\ 9} = 10.12$, $P < 0.05$; Table 3). Leaf number was similar between control seedlings and those in the 2% treatment. No leaves were produced in seedlings in the 3.5% treatment at 46 days (Table 3).

## 4. Discussion

Diesel-contaminated soil at 5%, 10% and 15% had a negative effect on photosynthesis, transpiration and stomatal conductance of *Q. oleoides* seedlings. However, seedlings showed certain resistance and tolerance under 2% of soil pollution; it is interesting that photosynthesis rate and growth increased when were compared with

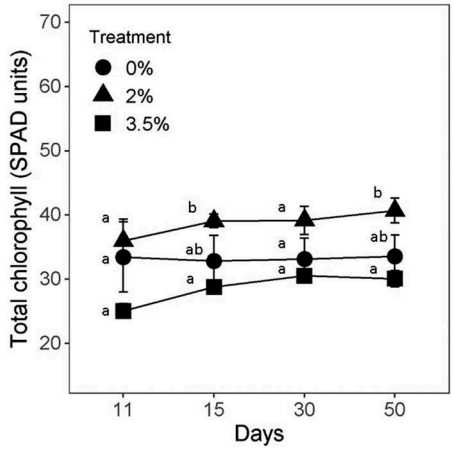


**Figure 6.** Total chlorophyll of *Quercus oleoides* under two levels of contamination by diesel (2% and 3.5%) and in control seedlings. Different letters mean significant differences among diesel treatment in each day ($P < 0.05$). Each point represents the mean ± SE ($n = 4$).

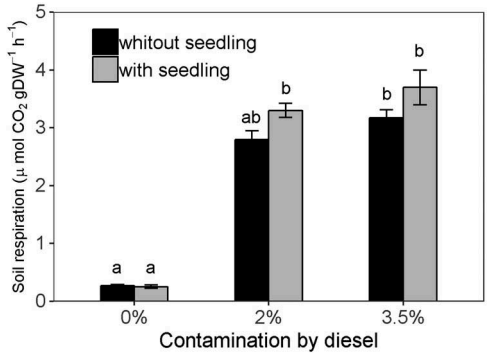


**Figure 7.** Soil respiration in treatments with 0%, 2% and 3.5% of soil diesel pollution. Different letters mean that the treatments are statistically different ($P < 0.05$). Each bar represents the mean ± SE ($n = 4$).

**Table 3.** Increase in height and leaf number of *Quercus oleoides* at different levels of diesel contamination at the beginning and at 46 days of experiment II.

| | Height (cm) | | | Leaf number | | |
|---|---|---|---|---|---|---|
| % of polluted | Start | End | Increase (cm) | Start | End | Increase of leaves number |
| 0 | 24.25 ± 3.7 | 26.5 ± 6.0* | 2.25 ± 1.2 | 11.5 ± 1.7 | 15.5 ± 6.8 | 4.00 ± 1.6 |
| 2 | 19.25 ± 7.4 | 23.2 ± 7.4* | 4.00 ± 0.07 | 9.2 ± 1.5 | 13.5 ± 2.5 | 4.25 ± 0.7 |
| 3.5 | 20.30 ± 11 | 20.2 ± 1.0* | 0 | 9.6 ± 4.4 | 3 ± 0 | 0 |

$n = 4$, ns: non-significant.
*Significant effect, $P < 0.001$.

control seedlings. Hydrocarbons such as diesel frequently have a negative effect on plant growth and it may even lead to death because of disintegration of the cell wall, reduction of gas exchange levels, chlorosis and other processes (Lin et al., 2002; Reynoso-Cuevas et al., 2008). Plants tolerance to pollutants may be very variable and it depends on the plant species, as well as the actual concentration of the pollutant in the soil (Adam & Duncan, 2002; Reynoso-Cuevas et al., 2008; Rivera-Cruz & Trujillo-Narcía, 2004). Harvey et al. (2001) mention that pollutant-related stress on plants may diminish cells ability to obtain reducing power and as a consequence the formation of reactive oxygen species. These may cause oxidation stress in cells, modifying metabolic activities such as photosynthesis. It has also been reported that light and aromatic fractions (present in diesel) are the most phytotoxic (Chaîneau et al., 1997). In that respect, Mallakin et al. (2002) found that anthracene, an aromatic fraction hydrocarbon with a medium fraction similar to that of diesel and its derivate, produced by photodecomposition, induces general damage to photosystems I and II of plants reducing photosynthesis rate.

Seedlings exposed to 5%, 10% and 15% pollution treatments showed lower photosynthesis activity when compared to control seedlings after 3 days. These seedlings could be in an acclimatization phase to contaminated soils, because seedlings exposed to the 5% of diesel-contaminated soil had the same photosynthetic responses at 3 and 9 days of treatment. The low values of stomatal conductance and transpiration rate at 3 and 9 days indicate that seedlings were getting adjusted in order to prevent further loss of water, which is an acclimation mechanism to stress (Li, 1991). In fact, seedlings that growth under 5% at 16 days registered a decrease in their RWC in comparison with seedlings control. This can be attributed to hydrophobic properties of diesel which reduce water infiltration and humidity of the soil (Merkl et al., 2005). In general, when plants are exposed to any kind of stress, they react by reducing their physiological processes, like photosynthesis and growth (Jahan et al., 2014; Lambers et al., 1998). If the plant enters the resistance stage, the changes that occur allow it to reach a new suboptimal physiological state for the current conditions, which corresponds to the maximum tolerance degree that plants may reach under stress conditions (Ashraf & Foolad, 2007). Therefore, it is important to know the physiological mechanisms that allow them to survive under suboptimal growth conditions. It is likely that the results related to a reduction of stomatal conductance, transpiration and RWC levels of *Q. oleoides* under the different diesel-contaminated soil treatments may be attributed to the toxicity and hydrophobicity of diesel in the soil as well as water infiltration to surface rhizosphere and to deeper soil levels are reduced substantially (Khairi et al., 2015; Labud et al., 2007).

The 2% (20,000 mg $kg^{-1}$) treatment stimulated growth of seedlings of *Q. oleoides*, which was unexpectedly higher than those under the 3.5%, even higher than control seedlings. These results are in agreement with the photosynthetic rate and transpiration showed by seedlings in the 2% treatment. This physiological response is known as hormesis, which is characterized by the stimulating effects shown at low exposition levels of toxic agents and inhibition when high doses are used (Forbes, 2000; Labra-Cardón et al., 2012; Roosens et al., 2003). Stomatal conductance of seedlings under 2% treatment registered twofold at the last days of experiment driving in an increase of both photosynthesis and growth, while that stomatal conductance of seedlings control and those under 3.5% treatment remained similar. Even though stomatal conductance in these later seedlings did not diminish, and transpiration registered a little decrease at the end of the experiment, how it goes with *Avicennia marina* and *Bruguiera gymnorhiza* have shown no differences in leaf stomatal conductance and transpiration between oiled treatments and controls (Naidoo et al., 2010). Diesel-contaminated soil have negative effects on the growth of *Spartina foliosa*, although 15% of plants survived in a diesel treatment, because diesel refined nature and low weight molecular (typically consists of compounds with 10–24 carbon atoms) make it more bio-available to the plants (Lam, 2012; Redondo-Gomez et al., 2014; Wilkinson et al., 2002). This response could explain in part the increase of photosynthesis and growth in seedlings of *Q. oleoides* at 2% of diesel-contaminated soil.

Seedlings of *Q. oleoides* exposed to 2% and 5% of diesel-contaminated soil significantly increased their total chlorophyll in comparison with control seedlings at 50 and 16 days, respectively. The increase of total chlorophyll in seedlings at 2% is related to their increase in photosynthetic activity, because chlorophyll is an important molecule in the light energy absorption and transformation in photosynthesis. This response supports the hormesis phenomenon in *Q. oleoides*, where plants tolerate low levels of diesel-contaminated soil. On the other hand, several works showed a decrease in chlorophyll content in plants as a response of direct toxic effects due to hydrocarbons pollution (Chaîneau et al., 1997; Achuba 2006; Li, Wang et al., 2008; Redondo-Gomez et al., 2014; Han et al., 2016).

Our results were similar to those observed for *Mimosa pigra* and *Cyperus elegans* (Rivera-Cruz et al., 2005), where low pollutant concentrations showed an increase in growth for these species and inhibition at higher doses. In addition, low concentration of hydrocarbon in soils stimulates growth of cotton plants due to the increase in carbon content (Plice, 1948). Another plants as the ornamental *Mirabilis jalapa* L. can effectively promote the degradation of total petroleum hydrocarbons when the concentration in the soil is equal to and lower than 10,000 mg $kg^{-1}$ (Peng et al., 2009); even, the fungi population in soil under 10,000 mg $kg^{-1}$ of petroleum hydrocarbons is greater than that in clean soil. Our results are contrasting with many reported for several plant species where some of the stress factors such as lack of nutrients, water availability and light or dark excess induce a reduction of growth rates and nutrient intake (Chapin, 1991). The stimulating growth effects on seedlings of *Q. oleoides* under soils with low pollutant require further research on their relevant mechanisms.

Hydrocarbon-polluted soils have been reported to have a negative growth response in legumes such as *Calopogonium mucunoides, Centrosema brasilianum* and *Stylosanthes capitata*. These species died at 42 and 56 days from exposure to raw oil (50,000 mg $kg^{-1}$, Merkl et al., 2005). These results are similar to the obtained for *Q. oleoides* at higher diesel concentrations than 50,000 mg $kg^{-1}$. It is suggested that at this concentration, seedlings of *Q. oleoides* have no defense mechanisms and the stressing factor may be higher than the plant ability to respond (Lambers et al., 1998).

The different light levels used in pollution treatments with *Q. oleoides* seedlings showed no effect on the photosynthetic activity. However, we found a little increase of stomatal conductance and transpiration at 200 µmol $m^{-2}$ $s^{-1}$ with a decrease of photosynthesis in comparison with other light levels at 15 and 30 days. At low light, microenvironment conditions such as temperature leaf and vapor pressure deficit leaf were slightly lower (26 ± 0.13°C; 1.64 ± 0.02 kPa, respectively) than other light levels (29 ± 0.11°C; 2.01 ± 0.02 kPa, respectively), which could mean a limit for assimilation of $CO_2$, because assimilation, activity of the Rubisco and electron transport depend on irradiance (Farquhar & Sharkey, 1982), although these responses need to be explored in detail. Seedlings of *Q. oleoides* could have the ability to grow either under shadow or open canopy without modifying their photosynthetic rate (Pimienta & Ramírez, 2003). However, it has been reported that their congeneric *Q. petraea* and *Q. pyrenaica* are susceptible to dryness when growing under a dense canopy of old pines, and their growth is retarded when compared to seedlings growing in open pines canopy (Rodríguez-Calcerrada, 2007). Light and water availability effect on seedlings physiology is useful information required in order to design reforestation and afforestation strategies as it provides important information on ecological requirements of the species (Kollmann & Grubb, 1999; Murata et al., 2007; Quero et al., 2006; Sack & Grubb, 2002).

Soil respiration is a relevant parameter to know the microbial degradation as well as the determination of dehydrogenase activity, among other techniques (Waarde et al., 1995). In this sense, soil respiration was proportional todiesel-contaminated soil, and was higher in soil with than without seedlings of *Q. oleoides*. The increase of soil respiration as a result of biological activity during the phase of the highest diesel oil degradation activity has been found by other authors (Atlas & Bartha, 1992; Margesin & Schinner, 1997). Hydrocarbon is conformed at most by carbon, and microorganisms can use it as an energy source increasing their metabolic activity (Abaye & Brookes, 2006; Adam & Duncan, 2002; Nilsson et al., 2005). Plants stimulate organic pollutant degradation, mainly to provide optimal conditions for the microbial diversity in the rhizosphere (Kruger et al., 1997; Peña-Castro et al., 2006). Hydrocarbons deposited in the soils modify the microbial populations, which inhibit plant coverage (Adams & Morales-García, 2008); furthermore, they induce catabolic microbial ways by compounds of vegetable origin (Francova et al., 2004; Siciliano & Germida, 1998; Top & Springael, 2003). Microbial degradation of oil-contaminated soil is a complex process and the biotic and abiotic factors have a great influence on the fate of spilled oil; therefore, it is quite necessary to test the rhizosphere microbial communities for increasing diesel degradation about the plant.

*Q. oleoides* resulted highly vulnerable to diesel-polluted soils at levels higher than 5%; but it keeps its

photosynthetic activity and growth in diesel-polluted soils at 2%, showing hormesis phenomenon. These results suggest that *Q. oleoides* could grow on soils that have been contaminated with low levels of diesel contamination. Notwithstanding, more field essays are needed in order to evaluate physiological responses of this species in soils with different pollutant concentrations and exposure times, and their capacity as a potential use for phytoremediation in contaminated soils. The results obtained are important since cultivation of a hydrocarbon soil-polluted tolerant species is a determinant condition in order to succeed in a phytoremediation process (Davis et al., 2002).

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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