

Influence of the hydrocarbons diesel, gasoline, and benzene on the growth and mineral and antioxidant concentrations of tomato plants

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Abstract

The produced water is obtained during the extraction process of hydrocarbons, whose characteristics, composition and concentration depend on the reservoir that contains them. The waters produced contain hydrocarbons and heavy metals, and may contain essential elements for plant nutrition. Some studies indicate that for plants the most toxic components of the produced water are the hydrocarbons. This research aimed to evaluate the response in the pH and the electrical conductivity (EC) of irrigation leachate, morphological variables, mineral concentration and the generation of antioxidants in the tomato plants treated with diesel, gasoline and benzene in concentrations of 15 and 30 mg L⁻¹, simulating the use of water produced for irrigation. An analysis of variance and tests of means of least significant difference was performed. The hydrocarbon treated plants reached the fifth cut of ripe fruits, except the treatment of diesel at 30 mg L⁻¹, in which only 45% of the plants survived, and only the first harvest of ripe fruits was obtained. According to their type and concentration, the hydrocarbons produced both favourable and unfavourable changes in the pH, EC, stem diameter, plant height and dry fruit weight. Also, the hydrocarbons produced both beneficial and detrimental changes in the mineral concentration of the plants; however, the hydrocarbons inhibited the mineral concentration in the fruits. The level of ascorbate in the fruits was decreased, and the diesel treatments limited the accumulation of lycopene.

Keywords: congenital waters; hydrocarbons pollution; nutritional quality; produced waters; soil pollution; *Solanum lycopersicum* L; water pollution

Introduction

The hydrocarbon deposits contain congenital water (Veil, 2015), which is the main waste (Martel-Valles *et al.*, 2013) obtained during the extraction process of hydrocarbons (Deng *et al.*, 2005). The extracted

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congenital water, named produced water, contains different composition and concentration (Manfra *et al.*, 2010) of mineral salts, suspended solids, heavy metals, microorganisms, organic and inorganic compounds (Head *et al.*, 2003). By its origin, the produced water contains hydrocarbons (ARPEL, 2012), that can cause unfavorable responses in living beings (Manfra *et al.*, 2010).

Hydrocarbons are a complex mixture of chemical compounds, mainly constituted by carbon and hydrogen atoms, and can be found in solid, liquid and gaseous states (Vallejo *et al.*, 2005). The total petroleum hydrocarbons (TPH) term is used to denote substances derived from crude oil (ATSDR, 1999). Hydrocarbons are classified according to the number of carbon atoms they contain, such as heavy fraction hydrocarbons (HFH), medium fraction hydrocarbons (MFH) and light fraction hydrocarbons (LFH), which are contained in crude oil, diesel, and gasoline and benzene, respectively (NOM-138-SERMARNAT/SSAI-2012, 2013).

Due to its natural origin, hydrocarbons can be reincorporated into the soil to be degraded in the biogeochemical cycles by microorganisms (Youssef *et al.*, 2009). When the amount of hydrocarbons in the environment is higher than the amount that can be metabolized, these become pollutants (Ramana-Rao *et al.*, 2012) with adverse effects in the soil biota (Pardo-Castro *et al.*, 2004), and delayed germination, decreased biomass and leaf area, and can cause necrosis and death in the plants (Adam and Duncan, 2002).

Martel-Valles *et al.* (2013) used produced water in tomato plants grown under greenhouse conditions, which was diluted with the irrigation water to reduce the electrical conductivity (EC), and to avoid the toxic effects of the water salinity (FAO, 1994). The results showed that only the treatment with high concentrations of MFH, Cu^{2+} and Cl^- , negatively affected the stem diameter, the leaf dry weight, the root length, and limited the mineral absorption causing the death of more than half of the evaluated plants; on the other hand, the fruits did not accumulate MFH or volatile hydrocarbons, such as the BTEX.

Many plant species are sensitive to petroleum contaminants (Wang *et al.*, 2003), and environmental pollution is a latent risk due to the use of hydrocarbons, such as gasoline and diesel fuels (Vallejo *et al.*, 2005). This research aimed to apply diesel, gasoline and benzene in the substrate of tomato plants through the irrigation water in concentrations of 15 and 30 mg L^{-1} , to evaluate the effect of these compounds on the pH and EC of the irrigation leachate, as well as on the morphological variables, the mineral concentration, the activity of catalase, and the ascorbate and lycopene concentrations in the tomato plants. The lower hydrocarbon concentration corresponded to the daily maximum limit for discharge into freshwater bodies, according to the Mexican Official Standard NOM-143-SEMARNAT-2003 (2005).

Materials and Methods

Crop establishment

The experiment was established into a plastic cover greenhouse in the Autonomous Agricultural University Antonio Narro in Saltillo, Mexico (25° 22' North, 101° 00' West, altitude 1760 m). Seeds of a tomato (*Solanum lycopersicum* L.) cv. 'Rio Grande' with determinate growth habits were seeded in polystyrene trays. After 38 days, the seedlings were transplanted in black polyethylene containers (16 L) filled with a mixture of peat moss and perlite (1:1 v/v). The crop cycle extended from March 13th to August 4th, 2014.

Crop development and management

Watering was applied three times a day (09:00, 13:00 and 18:00 h) through a drip irrigation system with high flow stakes on each tomato plant container, with a total volume of 1.5 $\text{L plant}^{-1} \text{day}^{-1}$. The first two irrigations consisted of a Steiner nutrient solution (Steiner, 1961) in a concentration of 25, 50, 75 and 100% at the 16, 38, 56 and 80 days after transplanting (DAT), respectively, while the third watering consisted in applications of water only. The pH of the nutrient solution was maintained with sulfuric acid (H_2SO_4) to ~6.5, and also the recommended standard cultural work for tomato grown into greenhouse conditions, regarding both the pruning and the hanging-string guided system, were performed.

Hydrocarbon treatments

The treatments consisted in the application of diesel (PEMEX), gasoline (PEMEX) and benzene (FAGA Lab), corresponding to the medium (diesel) and light (gasoline and benzene) hydrocarbon fractions, according to the Mexican Official Standard NOM-138-SERMARNAT/SSAI-2012 (2013), in concentrations of 15 and 30 mg L⁻¹, where the lower concentration corresponded to the daily maximum limit established for freshwater receiving bodies, according to the Mexican Official Standard NOM-143-SEMARNAT-2003 (2005). The control treatment consisted of tap water applications. In the hydrocarbon treatments, the products were diluted separately in 500 mL of water per each plant and then applied to the pot near to the stem of the tomato plants on the third irrigation of the day. The applied volume remained constant during the experiment.

pH and EC measurements

The irrigation leachate on five potted plants randomly chosen by treatment was collected on a plastic container placed under each potted plant on a daily basis. The pH and EC of the irrigation leachate were measured one hour after the third watering during the period of application of the treatments, with a HI 98130 portable equipment (Hanna® Instruments Inc.).

Morphological variables measurements

The measurements of the plant height (from the stem base to the apical part) and the stem diameter (at the stem base) were performed in two random samples, that is, 1) 20 tomato plants in the flowering stage at the 38 DAT, and 2) 16 tomato plants in the fruiting stage at the 80 DAT. The leaf (LDW) and stem (SDW) dry weights were determined using four and five plants randomly chosen in the flowering and fruiting stages, respectively. The fruit dry weight (FDW) was obtained by collecting the ripe fruits (category 6, USDA, 1997) from five plants per treatment at the 80, 87, 94, 101 and 108 DAT. In each measurement case, the fresh matter (leaves, stems, and fruits) was measured separately with a digital balance, and the leaves, stems, and fruits were dehydrated separately in an oven at 60 °C for 72 h.

Mineral concentration quantifying

The mineral concentration of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) in the tissues of root, stem and leaf, was analyzed at the flowering (38 DAT) and fruiting (80 DAT) stages, in the same collected plants in which the dry weights were measured, and were expressed in mol kg⁻¹ for macronutrients and mmol kg⁻¹ for micronutrients. N concentration was determined with the micro-Kjeldahl method (AOAC, 1980). P concentration was analyzed by the spectrophotometric method with aminonaphthol sulfonic acid reagent (Harris and Popat, 1954), using a Helios UV-Vis spectrophotometer (Thermo Electron Corporation) at 640 nm. The root, stem, leaf and fruit samples were dehydrated and subjected to acid digestion (AOAC, 1980) and subsequently the concentrations of K, Ca, Mg, Na, Fe, Mn, Zn and Cu, were analyzed with a Varian 920LC atomic absorption spectrophotometer (Varian Inc.). The mineral concentrations in the fruits were determined in five ripe fruits per treatment in the first cut (80 DAT), with the same techniques used for roots, stems, and leaves.

Antioxidant quantifying

The antioxidant concentrations in the leaves were determined in four leaves with physiological maturity per treatment, collected at the 80 DAT. The antioxidants in the fruits were determined in five ripe fruits per treatment at the 80 and 108 DAT. The procedures to obtain the extracts and quantify the enzymatic activity of catalase, were performed according to Ortega-Ortiz *et al.* (2007). The ascorbate and lycopene were determined by titration and colorimetry, respectively, according to García-Enciso *et al.* (2014).

Data analysis

The hydrocarbon treatments and the control treatment were set up into the greenhouse on a completely randomized design with 20 one-potted plant replications. Data were analyzed with analysis of variance (ANOVA) and a multiple mean comparison test according to Fisher least significant difference (Fisher LSD, $P \leq 0.05$) was performed with the R program (R Core Team, 2019).

Results*pH and EC of the irrigation leachate*

The pH and EC of the irrigation leachate of the tomato plants showed significant differences ($P \leq 0.05$) in comparison with control treatments. The EC increased with the three hydrocarbons, while the pH decreased in the benzene and gasoline treatments, and increased with the diesel application (Table 1). As the hydrocarbon concentration increased from 15 to 30 mg L⁻¹, the pH and EC decreased with benzene and increased with gasoline, and showed an antagonistic behavior with diesel, reflected in a significant accumulation of salts and slight acidification.

Table 1. Mean values of pH and EC of the irrigation leachate of tomato plants treated with hydrocarbons

Treatment (mg L ⁻¹)	pH	EC (dS m ⁻¹)
Control	6.14 ab	1.16 d
Diesel 15	6.26 a	1.45 b
Diesel 30	6.18 ab	1.56 a
Gasoline 15	5.90 bc	1.18 d
Gasoline 30	6.06 c	1.43 bc
Benzene 15	6.05 bc	1.32 c
Benzene 30	5.54 d	1.17 d

Morphological variables

The morphological variables showed significant differences ($P \leq 0.05$) respect to the corresponding control treatments, only in the plant height and stem diameter in the flowering stage (Figure 1), and in the stem diameter in the fruiting stage (Figure 2). In the flowering stage (38 DAT), the plant height was 9% lower with benzene and 7.7% lower with diesel. The benzene concentration had opposite effects on the stem diameter, with a decrement of 12.1% and an increment of 14.7% in the 15 and 30 mg L⁻¹, respectively. In the fruiting stage (80 DAT), the stem diameter increased by 12.7% with the application of benzene at 30 mg L⁻¹. The diesel treatments significantly decreased the plant height as the concentration increased from 15 to 30 mg L⁻¹, and only 45% of the plants irrigated with the higher level survived until the first harvest of fruits.

Mineral concentration in the flowering stage

In the flowering stage (38 DAT) the mineral concentration was significantly ($P \leq 0.05$) modified in the leaves and stems, and improved in the roots respect to the corresponding control treatments, with the hydrocarbon application in the tomato plants. Regarding the leaves, the Na increased 50% with benzene at 15 mg L⁻¹. The Mn decreased with the three hydrocarbons: 26.4% with benzene at 15 mg L⁻¹, 43.1% with diesel at 30 mg L⁻¹, and 38.2% with gasoline. The Zn decreased 42.3% with benzene at 30 mg L⁻¹ and 46.2% with diesel at 15 mg L⁻¹. Concerning the stems, the P increased 46.3 and 51.7% in the lower hydrocarbon doses with benzene and gasoline, respectively. The Na increased more than twice with benzene at 15 mg L⁻¹. The Fe increased 55.3% with gasoline at 15 mg L⁻¹. The Mn decreased 44.6 and 45.8% in the higher hydrocarbon doses with diesel and gasoline, respectively. Respecting the roots, the Cu increased 24.6 and 26.9% in the higher hydrocarbon doses with diesel and gasoline, respectively (Table 2).

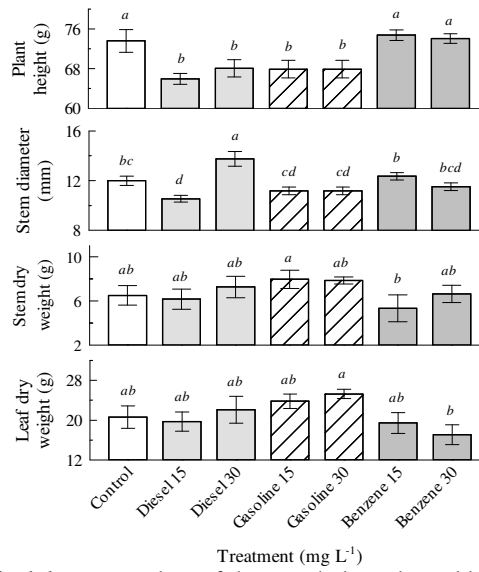


Figure 1. Mean and standard deviation values of the morphological variables in the flowering stage (38 DAT) in tomato plants treated with hydrocarbons. Different letters between treatments denote significant differences (Fisher's LSD test, $P \leq 0.05$). $n = 4$ for leaf and stem dry weights, $n = 20$ for stem diameter and plant height.

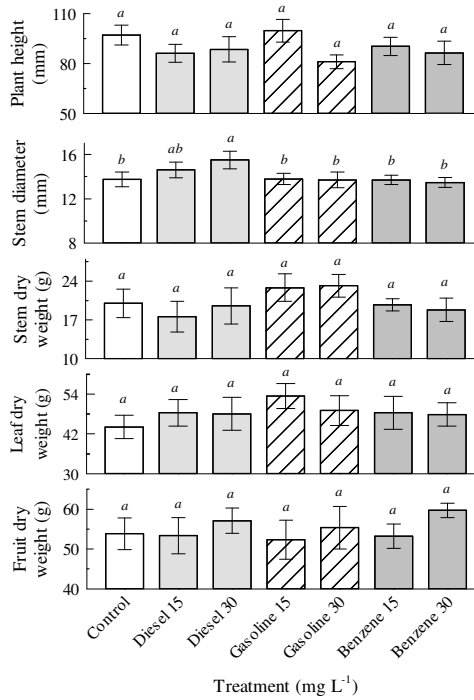


Figure 2. Mean and standard deviation values of the morphological variables in the fruiting stage (80 DAT) in tomato plants treated with hydrocarbons. Different letters between treatments denote significant differences (Fisher's LSD test, $P \leq 0.05$). $n = 16$ for stem diameter and plant height, $n = 4$ for leaf and stem dry weights, $n = 5$ for fruit dry weight.

Table 2. Mean values of the mineral concentration in the flowering stage (38 DAT) of tomato plants treated with hydrocarbons

Treatment (mg L ⁻¹)	Macronutrients (mol kg ⁻¹)						Micronutrients (mmol kg ⁻¹)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
	Leaf									
Control	2.31 ab	0.19 a	0.80 a	0.88 a	0.24 a	0.08 b	2.36 a	1.47 a	1.26 ab	0.08 a
Diesel 15	2.18 ab	0.19 a	0.81 a	0.82 a	0.21 a	0.06 b	2.07 a	1.15 abc	0.68 c	0.10 a
Diesel 30	2.40 ab	0.17 a	0.51 a	0.76 a	0.24 a	0.07 b	1.96 a	0.84 c	0.92 bc	0.09 a
Gasoline 15	2.54 ab	0.20 a	0.64 a	0.76 a	0.27 a	0.07 b	1.99 a	0.91 c	1.13 ab	0.10 a
Gasoline 30	2.62 a	0.20 a	0.68 a	0.75 a	0.24 a	0.07 b	1.80 a	1.03 bc	1.10 ab	0.09 a
Benzene 15	2.57 a	0.22 a	0.77 a	0.88 a	0.26 a	0.12 a	1.92 a	1.08 bc	1.41 a	0.08 a
Benzene 30	1.91 b	0.16 a	0.68 a	0.76 a	0.23 a	0.07 b	2.12 a	1.32 ab	0.73 c	0.09 a
	Stem									
Control	1.52 ab	0.15 c	0.89 a	0.34 a	0.14 a	0.06 b	0.97 b	0.42 a	1.67 a	0.08 a
Diesel 15	1.67 ab	0.20 abc	0.85 a	0.31 a	0.14 a	0.06 ab	0.89 b	0.32 ab	1.70 a	0.06 a
Diesel 30	1.25 b	0.17 abc	0.87 a	0.34 a	0.14 a	0.06 b	1.01 ab	0.23 b	1.77 a	0.08 a
Gasoline 15	1.75 a	0.22 a	1.02 a	0.29 a	0.14 a	0.05 b	1.50 a	0.31 ab	1.65 a	0.09 a
Gasoline 30	1.52 ab	0.19 abc	0.97 a	0.50 a	0.12 a	0.04 b	1.16 ab	0.23 b	1.45 a	0.10 a
Benzene 15	1.51 ab	0.22 ab	0.99 a	0.29 a	0.15 a	0.16 a	1.06 ab	0.40 a	1.88 a	0.08 a
Benzene 30	1.29 ab	0.16 bc	0.91 a	0.33 a	0.14 a	0.07 ab	1.27 ab	0.36 a	1.60 a	0.09 a
	Root									
Control	1.08 ab	0.13 a	0.56 a	0.33 a	0.15 a	0.16 a	10.72 a	0.54 a	1.18 a	0.13 cd
Diesel 15	1.07 ab	0.11 a	0.47 a	0.35 a	0.15 a	0.15 a	8.73 a	0.56 a	1.27 a	0.13 cd
Diesel 30	1.04 ab	0.11 a	0.47 a	0.35 a	0.13 a	0.14 a	11.26 a	0.42 a	1.42 a	0.16 ab
Gasoline 15	1.24 ab	0.11 a	0.49 a	0.32 a	0.14 a	0.16 a	11.92 a	0.60 a	1.32 a	0.15 abc
Gasoline 30	1.20 ab	0.10 a	0.46 a	0.37 a	0.13 a	0.13 a	10.36 a	0.65 a	1.40 a	0.17 a
Benzene 15	0.98 b	0.14 a	0.64 a	0.38 a	0.12 a	0.12 a	5.40 a	0.39 a	1.53 a	0.13 bcd
Benzene 30	1.34 a	0.09 a	0.63 a	0.55 a	0.19 a	0.16 a	6.14 a	0.37 a	1.24 a	0.12 d

Note: Different letters between treatments denote significant differences (Fisher's LSD test, $P \leq 0.05$). $n = 20$.

Mineral concentration in the fruiting stage

In the fruiting stage (80 DAT), hydrocarbon application modified ($P \leq 0.05$) the mineral levels in the leaves, improved the values in the stems and roots, and diminished the levels in the fruits compared to the control treatments. Regarding the leaves, the P decreased by 32.6% with benzene. The Fe increased 62.3% with diesel at 30 mg L⁻¹. The Cu increased 36.7% with benzene at 15 mg L⁻¹ and 32.7% with diesel. Concerning the stems, the Ca increased 92.8% with gasoline at 15 mg L⁻¹. The Na increased 36.4% with diesel at 30 mg L⁻¹. Respecting the roots, the P increased 68.5% with diesel at 15 mg L⁻¹. The Fe and Mn increased more than twice in the lower hydrocarbon doses with gasoline and diesel, respectively. Regarding the fruits, the P decreased by 16.7% with gasoline at 30 mg L⁻¹. The Ca fell by half with diesel at 30 mg L⁻¹. The Na decreased 35.7 and 46.4% with diesel at 30 mg L⁻¹ and gasoline, respectively. The Cu decreased by 19.8% with diesel at 30 mg L⁻¹ (Table 3).

Antioxidant concentration

Hydrocarbon application in the tomato plants improved ($P \leq 0.05$) the levels of antioxidants in the leaves; on the other hand, the antioxidants diminished in the fruits. Regarding the leaves, the ascorbate increased 40.7% with benzene at 30 mg L⁻¹, and decreased 36% with diesel at 30 mg L⁻¹. The catalase increased more than twice in the higher doses with diesel and gasoline. Concerning the fruits, at the 80 DAT the ascorbate decreased 42.9% with benzene at 30 mg L⁻¹, 48.6% with diesel at 15 mg L⁻¹, and 34.6% with gasoline at 15 mg L⁻¹. At the 108 DAT the lycopene decreased 38.2 and 31.1% in the lower doses with diesel and gasoline, respectively.

The leaf ascorbate in the flowering stage (38 DAT), and the fruit lycopene in the fruiting stage (108 DAT) showed no significant differences ($P > 0.05$) respect to the corresponding control treatments. The

ascorbate and lycopene measurements at the 80 DAT in the fruits of tomato plants treated with diesel were not performed, due to the toxicity of this hydrocarbon at 30 mg L⁻¹ inhibited more than half of the plant's growth and development (Table 4).

Table 3. Mean values of the mineral concentration in the fruiting stage (80 DAT) of tomato plants treated with hydrocarbons

Treatment (mg L ⁻¹)	Macronutrients (mol kg ⁻¹)						Micronutrients (mmol kg ⁻¹)			
	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
	Leaf									
Control	2.11 ab	0.40 a	0.70 ab	1.44 ab	0.33 a	0.02 a	2.26 b	3.09 a	0.39 a	0.10 c
Diesel 15	2.14 a	0.34 ab	0.91 a	1.37 ab	0.32 a	0.04 a	3.23 ab	3.14 a	0.38 a	0.13 a
Diesel 30	1.76 ab	0.31 ab	0.76 ab	1.50 a	0.29 a	0.03 a	3.67 a	3.17 a	0.42 a	0.13 ab
Gasoline 15	2.02 ab	0.31 ab	1.04 a	1.12 b	0.26 a	0.04 a	2.39 b	2.57 a	0.65 a	0.10 c
Gasoline 30	1.86 ab	0.30 ab	0.84 a	1.26 ab	0.31 a	0.04 a	2.67 ab	3.17 a	0.42 a	0.10 bc
Benzene 15	2.09 ab	0.29 b	0.44 b	1.36 ab	0.34 a	0.13 a	2.29 b	3.22 a	0.43 a	0.13 a
Benzene 30	1.65 b	0.27 b	0.78 ab	1.41 ab	0.36 a	0.02 a	2.57 ab	2.89 a	0.46 a	0.12 abc
	Stem									
Control	1.15 a	0.31 a	0.75 a	0.33 b	0.19 ab	0.03 bcd	1.18 a	0.78 ab	1.76 a	0.14 a
Diesel 15	1.19 a	0.29 a	0.78 a	0.36 ab	0.13 b	0.03 cd	1.05 a	0.75 ab	1.54 a	0.13 a
Diesel 30	0.92 a	0.29 a	0.90 a	0.39 ab	0.16 ab	0.05 a	1.06 a	0.55 b	1.68 a	0.07 b
Gasoline 15	1.11 a	0.29 a	0.86 a	0.64 a	0.23 a	0.04 abc	1.50 a	1.68 a	1.95 a	0.15 a
Gasoline 30	0.75 a	0.26 a	0.75 a	0.36 b	0.13 b	0.04 ab	0.92 a	0.64 b	1.54 a	0.11 ab
Benzene 15	1.01 a	0.30 a	0.79 a	0.38 ab	0.18 ab	0.03 d	1.26 a	0.82 ab	1.90 a	0.15 a
Benzene 30	0.90 a	0.23 a	0.92 a	0.43 ab	0.16 ab	0.03 d	1.18 a	0.81 ab	2.07 a	0.15 a
	Root									
Control	0.93 ab	0.11 bc	0.34 a	0.60 a	0.11 abc	0.11 a	2.47 b	0.34 b	1.36 ab	0.14 a
Diesel 15	0.95 a	0.19 a	0.43 a	0.68 a	0.11 abc	0.11 a	3.10 b	1.20 a	1.83 a	0.39 a
Diesel 30	0.85 ab	0.17 ab	0.30 a	0.67 a	0.08 c	0.14 a	3.84 b	1.08 ab	1.05 b	0.18 a
Gasoline 15	0.97 a	0.11 bc	0.36 a	0.48 a	0.12 abc	0.13 a	14.88 a	0.53 ab	1.00 b	0.17 a
Gasoline 30	0.63 b	0.06 c	0.38 a	0.54 a	0.09 bc	0.06 a	4.83 b	1.05 ab	0.82 b	0.14 a
Benzene 15	0.76 ab	0.10 bc	0.64 a	0.61 a	0.14 ab	0.13 a	1.54 b	0.38 b	0.92 b	0.14 a
Benzene 30	0.85 ab	0.12 abc	0.60 a	0.60 a	0.16 a	0.13 a	3.42 b	0.63 ab	1.29 b	0.21 a
	Fruit									
Control	1.34 a	0.20 a	0.94 a	0.05 ab	0.06 ab	0.03 a	1.33 a	0.18 a	0.53 ab	0.13 a
Diesel 15	1.41 a	0.20 a	0.88 a	0.05 a	0.05 b	0.03 a	1.22 a	0.19 a	0.53 ab	0.12 ab
Diesel 30	1.58 a	0.20 a	0.90 a	0.02 c	0.05 b	0.02 b	1.12 a	0.15 a	0.58 ab	0.10 b
Gasoline 15	1.25 a	0.20 a	0.82 a	0.05 a	0.07 a	0.02 b	1.28 a	0.21 a	0.63 ab	0.11 ab
Gasoline 30	1.40 a	0.17 b	0.90 a	0.05 ab	0.05 b	0.02 b	1.14 a	0.19 a	0.58 ab	0.11 ab
Benzene 15	1.24 a	0.21 a	0.96 a	0.04 b	0.05 b	0.03 a	1.07 a	0.17 a	0.44 b	0.12 ab
Benzene 30	1.31 a	0.20 ab	0.89 a	0.05 a	0.05 b	0.03 a	1.34 a	0.14 a	0.72 a	0.12 ab

Note: Different letters between treatments denote significant differences (Fisher's LSD test, $P \leq 0.05$). $n = 4$ for leaf, stem and root, $n = 5$ for fruit.

Table 4. Mean values of the antioxidant concentration in the tomato leaves and fruits of tomato plants treated with hydrocarbons

Treatment (mg L ⁻¹)	Leaf			Fruit		
	Ascorbate (mg g ⁻¹)	Ascorbate (mg g ⁻¹)	Catalase (mM min ⁻¹ g ⁻¹)	Ascorbate (mg g ⁻¹)	Lycopene (μg g ⁻¹)	Lycopene (μg g ⁻¹)
	38 DAT		80 DAT			108 DAT
Control	0.095 a	0.086 b	275 c	0.107 a	6.91 ab	8.24 a
Diesel 15	0.095 a	0.068 bc	675 bc	0.055 c	7.05 ab	5.09 c
Diesel 30	0.110 a	0.055 c	900 b		5.17 c	
Gasoline 15	0.070 a	0.081 b	650 bc	0.070 c	6.30 abc	5.68 bc
Gasoline 30	0.084 a	0.090 b	1550 a	0.075 bc	5.79 bc	6.91 ab
Benzene 15	0.112 a	0.073 bc	400 bc	0.094 ab	7.60 a	7.21 ab
Benzene 30	0.088 a	0.121 a	500 bc	0.062 c	5.83 bc	6.98 ab

Note: Different letters between treatments denote significant differences (Fisher's LSD test, $P \leq 0.05$). $n = 4$ for leaf, $n = 5$ for fruit.

Discussion

pH and EC of the irrigation leachate

It is known that the hydrocarbons can directly modify the pH of aqueous solutions (Adams *et al.*, 2008). However, the effects observed in this study were probably related to the impact of hydrocarbons on the chemical properties of the nutrient solution, radical exudates, substrate pores and about the metabolism of the radical cells and the microorganisms associated with the root (Siciliano *et al.*, 2003; Atekwana *et al.*, 2004; Adams *et al.*, 2008).

There are different degrees of sensitivity of the biota to the hydrocarbons, according to their type and concentration (Manfra *et al.*, 2010). The standard NOM-143-SEMARNAT-2003 (2005) established the level of 15 mg L⁻¹ as the daily maximum permissible limit of hydrocarbons for the discharge of produced water in freshwater receiving bodies. Both the bioaccumulation and the toxicity of the hydrocarbons increase as the octanol-water partition coefficient (Kow) of the hydrocarbon increase, so that the regression of toxicity data versus the log Kow is a straight line, and the log Kow measurement allows to know the hydrocarbon toxicity (Battelle, 2007). The hydrocarbons effect in the environment depends partially on the partition coefficient (log Kow), e.g. if the hydrocarbon is more soluble in water, its toxic effect in the biota is less since it shows less association with the lipids of the cell membranes, and modifies the pH and EC possibly through its interaction with the ions of the soil or substrate solution. In this case the toxicity seems to be indirect, through the modification of the soil pore water or substrate solution (Hansch *et al.*, 1995; ATSDR, 1999; TOXNET, 2019a, 2019b and 2019c). Gasoline and benzene, with low log Kow values (1.81-4.45 and 2.13, respectively) (Battelle, 2007; TOXNET, 2019a and 2019c) at 15 and 30 mg L⁻¹ concentrations decreased the pH values with respect to the control treatment, probably as a consequence of its relatively high solubility. Another possibility to explain the decrease in the pH is offered by Henner *et al.* (1999), who reported that the benzene in high doses produces phytotoxic effects that cause stress, causing the plants to release H⁺ by the root, which makes the medium more acidic.

In contrast, the application of diesel in the two concentrations did not change the pH of the irrigation leachate, probably due to its higher partition coefficient (log Kow 3.3-7.06) (TOXNET, 2019b), which corresponds to low water solubility, but indicative of ease interaction with the cell membranes. The above may explain their high toxicity that inhibited the growth and development of the tomato plants treated with a higher concentration (30 mg L⁻¹). Most of the hydrocarbon treatments increased the EC in the irrigation leachate of the tomato plants, which could be due to the chemical interactions between the hydrocarbons and the dissolved ions, as well as the restricted ions absorption caused by toxicity in the roots (Henner *et al.*, 1999; Reynoso-Cuevas *et al.*, 2008).

Morphological variables

No unambiguous response to the concentration or the hydrocarbon type was found. The treatment with benzene at 30 mg L⁻¹ favorably modified the stem diameter; similar results were obtained by Martínez and López (2001) and Baher *et al.* (2002), where the hydrocarbons application stimulated the growth and production of the biomass. In contrast, a decrease in the plant height was found in both the two treatments with benzene, coinciding with Adam and Duncan (2002) and Martel-Valles *et al.* (2013 and 2014). The variations in the results obtained are difficult to explain due to the complexity of interactions between the hydrocarbons, ions dissolved in the soil or substrate solution, plant roots and associated microorganisms. Nowadays, as far as we know, there is no information available about the transcriptomic, metabolomic, biochemical, or microbiome profiles in the roots of the plants grown in a medium with hydrocarbon pollution. These studies would be useful to explain the responses of the plants to different types and concentrations of hydrocarbons.

Mineral concentration

Previous studies agree that the hydrocarbons produce changes in the plant mineral distribution (Martel-Valles *et al.*, 2013) and that these changes do not show a direct relationship between the concentration and type of hydrocarbon with the mineral absorption of the plant (Martínez and López, 2001). In the present study, according to its nature and concentration, the hydrocarbon treatments produced positive and negative changes in the mineral concentration in the tissues of the plant organs. At the 38 DAT in the leaves, the benzene application at 15 mg L⁻¹ elevated the Na concentration, while the Mn concentration was affected by the three hydrocarbons, and the Zn concentration was also reduced with the benzene and diesel treatments. In the stems, the benzene application at 15 mg L⁻¹ raised the P and Na concentrations, and the gasoline treatment of 15 mg L⁻¹ improved the P and Fe concentrations, while the Mn concentration was affected by the diesel and gasoline treatments. In the root, the Cu concentration was favored by the higher applied doses of diesel and gasoline. At the 80 DAT in leaves, the Cu concentration was elevated with the benzene application at 15 mg L⁻¹ and diesel, and the Fe concentration was improved with diesel at 30 mg L⁻¹, while the P concentration was reduced with benzene. In the stems, the Ca and Na concentrations were raised with the gasoline application at 15 mg L⁻¹ and diesel 30 mg L⁻¹, respectively. In the root, the lower doses of diesel and gasoline improved the mineral concentrations of P and Mn, and Fe, respectively. It is known that plants that grow in environments polluted with hydrocarbons, develop a tolerance degree to specific chemical components of these (Hardoim *et al.*, 2008). Probably, while the plant makes necessary adjustments to build tolerance to the hydrocarbons, it modifies its ability to absorb, transport and assimilate certain minerals (Haydon and Cobbett, 2007; Glick and Stearns, 2011; Rohrbacher and St-Arnaud, 2016).

In the tomato fruits, the gasoline and diesel applications significantly affected the mineral concentration, and like to the root and stems, the benzene treatments did not affect the mineral concentration in the fruits. The P and Na concentrations reduced with the gasoline applications, while the Ca, Na, and Cu concentrations decreased with the higher diesel application. The hydrocarbon treatments, apparently limited the mobilization of the minerals towards the fruit, possibly due to the modification of some mechanism maintaining the homeostasis of the mineral concentration (Clemens and Ma, 2016), such as the carriers and transporters related to the absorption, translocation and partition processes of the minerals (Haydon and Cobbett, 2007).

Antioxidant concentration in the leaves

The hydrocarbons produced positive and negative changes in the leaf and fruit antioxidants, possibly due to the specific oxidizing stimuli of each combination of hydrocarbon type and concentration, which is a behavior that has been studied for other kinds of stress-inducing environmental stimuli (Meyer, 2008). The higher doses of the diesel and gasoline treatments increased the activity of catalase in the plant leaves at the 80 DAT; possibly the presence of the hydrocarbons increased the level of the lipid peroxidation, elevating the levels of reactive oxygen species that activate the enzymatic antioxidant system of the plants (Liu *et al.*, 2009).

On the other hand, the ascorbate concentration decreased with diesel treatment at 30 mg L⁻¹, coinciding with Silva-Doza *et al.* (2010), who found that after reaching the maximum activity of the ascorbate in the first six to nine days of exposure to stress, the progressive decrease of this antioxidant is a normal behavior. The ascorbate reduction as a response to the diesel application can be related to the higher toxicity of the MFH (Martel *et al.*, 2013 and 2014).

Antioxidant concentration in the fruits

In the fruit, the stressor agents produced changes in the lycopene and ethylene (Lurie and Klein, 1991), creating an apparent rapid response in the plant to the oxidative stress (Rodríguez-Verástegui *et al.*, 2016). However, if the stress is too intense, it can affect the response mechanisms of the plant (Lewis and Pryor, 2013), which is the possible reason why lycopene concentration in the fruit decreased with the diesel application at 15 mg L⁻¹ at the 80 DAT and with gasoline at 15 mg L⁻¹ at the 101 DAT, in addition to the death of the plants caused by the diesel application at 30 mg L⁻¹ at the 80 DAT. The reduction in the antioxidant generation is due to the hydrocarbon toxicity, that depending on their type and concentration (Esquivel-Cote *et al.*, 2013) produces stress-related changes in the plant (Haydon and Cobbett, 2007), and induces a premature maturity for each hydrocarbon concentration, which is a condition that decreases the antioxidant level (Zapata *et al.*, 2007).

Conclusions

In the irrigation leachates, the application of benzene at 30 mg L⁻¹ and gasoline at 15 mg L⁻¹ decreased the pH. Most of the hydrocarbon treatments increased the leachate's EC. The treatment with benzene at 30 mg L⁻¹ raised the stem diameter, while the treatments with benzene at 15 mg L⁻¹ and diesel at 30 mg L⁻¹ decreased the plant height. The higher concentration of diesel inhibited the biomass growth and caused the death of more than half of the tomato plants, which survived only until the first cut of ripe fruits. The mineral concentration in the roots, stems and leaves, showed positive and negative changes depending on the hydrocarbon type and concentration. In the fruit, most of the treatments limited the mineral concentration, mainly in the P and Na by the gasoline, and Ca, Na and Cu by the diesel. Regarding the antioxidant level, in the leaves, the catalase increased its activity with the diesel and gasoline treatments in the higher doses, and the ascorbate limited its concentration with the diesel treatment at 30 mg L⁻¹. In the fruit, the benzene at 30 mg L⁻¹ and diesel at 15 mg L⁻¹ limited the concentration of the ascorbate, and the lycopene decreased its activity with the diesel and gasoline in the lower doses.

Authors' Contributions

Conceptualization: ABM; Data curation: AMM, IM; Methodology: ABM, RFP; Writing - original draft: AMM, JFMV; Writing - review and editing: AMM, ABM.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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