Crude oil induces plant growth and antioxidant production in Leersia hexandra Sw. A hydrophytic grass that rhizodegrades oil in Tabasco, Mexico.

José Alberto Orocio-Carrillo
Colegio de Postgraduados Campus Tabasco
Maria del Carmen Rivera-Cruz (mariari@colpos.mx)
Colegio de Postgraduados Campus Tabasco https://orcid.org/0000-0003-4178-7252
Antonio Juárez-Maldonado
Universidad Autonoma Agraria Antonio Narro
Consuelo del Carmen Bautista-Muñoz
Colegio de Postgraduados Campus Tabasco
Antonio Trujillo-Narcía
Universidad Popular de la Chontalpa
Yolanda González-García
Universidad Autonoma Agraria Antonio Narro
Said Cárdenas-Villegas
Colegio de Postgraduados Campus Tabasco

Research Article

Keywords: Azotobacter, Hydrogen peroxide, Phenylalanine-ammonia-lyase, Phytoremediation, Total phenols

Posted Date: September 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1999247/v1

Abstract

This study evaluated the survival of the grass *Leersia hexandra* (Lh) to the stress caused by crude oil (CO), by means of synthesis of reactive oxygen species, antioxidants in leaf-root, and by the rhizospheric bacterial activity of Lh. The experiment was carried out for 180 days, a 4x2 factorial arrangement was applied, four concentrations of CO and two technologies [phytoremediation (PH) with Lh and natural attenuation (NA) with native microorganisms]. The results recorded treatment means with statistical differences (Duncan $p \leq 0.05$). Concentrations of 90 g/kg stimulated the formation of young plants (YP) and grass root. In YP leaves, the biosynthesis of H_2O_2, total phenols (TP) and total flavonoids (TFV) increased due to the effect of CO, as did catalase (CAT) and phenylalanine ammonia lyase (PAL) in roots. Grass roots with low H_2O_2 synthesis but high synthesis of CAT and PAL are a signal of tolerance to abiotic stress. Rhizosphere of Lh stimulated microbial activity and the population of *Azotobacter* spp but removal 22% de 90 g/kg the CO, and AN 58%. The results suggest adaptation of Lh to the stress caused by soil contaminated with oil, manifested by regulating the synthesis of H_2O_2 and antioxidants in leaf-root during the removal of hydrocarbons from oil.

Introduction

Crude oil (CO) contains aliphatic, aromatic and polar hydrocarbons or resins, as well as asphaltenes (Moubasher et al. 2015). Hydrocarbons are hydrophobic, recalcitrant, persistent, potentially carcinogenic, mutagenic and toxic to living beings (Logeshwaran et al. 2018). They also induce physical, chemical (Devatha et al. 2019) and biological changes in the soil. The evolutionary process of various wetland plant species has involved a process of adaptation to stress factors. The list of such plants is long: *Typha latifolia* (Omari et al. 2003), *Scirpus grossus* (Al-Baldawi et al. 2015), *Ludwigia octovalvis* (Al-Mansoory et al. 2017), *Leersia hexandra* (Orocio-Carrillo et al. 2019), *Phragmites australis* and *Typha domingensis* (Afzal et al. 2019), *Scirpus mucronatus*, *Cyperus laxus*, *C. esculentus* and *Ludwigia peploides* (Al-Mansoory et al. 2017). These plants have developed physiological and biochemical mechanisms that provide them with tolerance and resistance to various types of stress by inducing regulatory responses, homeostatic cellular restoration, or a reduction of phytotoxic effects (Mickelbart et al. 2015). Most studies of the effect of plant exposure to CO examine the relationship between dose and vegetative growth and biomass production. Only a few studies focus on the relationship between reactive oxygen species (ROS), antioxidants, growth, and production. H_2O_2 is an ROS species with a dual function inside plants. At low concentrations, H_2O_2 acts as a signal for acclimation and triggers tolerance to biotic and abiotic stress (Fukao and Bailey-Serres 2004; Laloi et al. 2004). At high concentrations, H_2O_2 induces programmed cell death (Dat et al. 2000), also called apoptosis. Cell death can occur as a result of the role played by H_2O_2 in physicochemical reactions that convert high-molecular-weight acids into lipid peroxide and degrade cell wall proteins and nucleic acids (Alanbary et al. 2019; Odukoya et al. 2019; Sachdev et al. 2021). Plant resistance to abiotic stress manifests in its foliage and root morphology, as well as in cell metabolism, including synthesis of H_2O_2 and antioxidant molecules (Soares et al. 2012; Siqueira-Soores et al. 2013). The main factors related to CO that are responsible for the self-regulation of phytotoxic processes, resistance and plant adaptation require further study. Plant biomarkers associated with oxidative stress have been reported before in studies on non-enzymatic antioxidants [ascorbic acid/ascorbate (AA), total phenol (TP), total flavonoids (TFV)], enzymatic antioxidants catalase (CAT), located mainly in the peroxisomes, mitochondria (del Río et al. 2006; Yap et al. 2021), phenylalanine ammonia lyase (PAL), which is produced from chorismate by the action of the dehydratase, and aminotransferase enzymes (Chen et al. 2006). Antioxidants counteract oxidative stress by protonating H_2O_2 in the electron transport chain during photorespiration (Halliwell 2006). Phytoremediation technology for the recovery of soil contaminated with CO is based on the use of plants that have adapted to contaminated soils and that produce biochemical biomarkers (ROS and antioxidants) in response to stress (Agathokleous and Calabrese 2020; Fan et al. 2021). They also form fibrous, extensive roots and support free-living and symbiotic microorganisms associated with the rhizosphere that oxidize, and reduce petroleum hydrocarbons (Rivera-Cruz et al. 2016; Zuzolo et al. 2021).

Lh is a grass that grows in oil-contaminated wetlands in southeastern Mexico. It has developed stress tolerance mechanisms that manifest in the hormetic response of its growth rate, in the increase of its plant biomass and crude protein content (Orocio-Carrillo et al. 2019). Lh also has a rhizosphere colonized by hydrocarbonoclastic bacteria and fungi (González-Moscoso et al. 2019). The adaptation of these microorganisms to the presence of crude oil suggests the presence of genes related to hydrocarbon degradation similar to those found in the bacteria *Pusillimonas*, *Dietzia*, *Bradyrhizobium*, *Azospirillum*, and *Azotobacter* (Arias-Trinidad et al. 2017; Wang et al. 2021). When associated with the roots of Lh, these bacteria stimulate the removal of TPH from the soil (Orocio-Carrillo et al. 2019). This study aims to increase our knowledge of why Lh grass, when exposed to CO under stress conditions, increases its growth rate and biomass production while removing CO from the soil. These questions can be answered through the study of the synthesis of ROS and the antioxidants that regulate metabolism under stress conditions. The objectives of the present study were: a) to determine the effect of CO on the biomass production of Lh and the formation of biochemical stress biomarkers (H_2O_2) and antioxidants (non-enzymatic and enzymatic) in leaves and roots; b) to analyze the effect of the rhizosphere of Lh and the rhizobacteria established on it on the removal of TPH and root antioxidants; c) to propose a biological technology for the recovery of soil contaminated with CO based on a native grass of the Mexican humid tropics adapted to the stress induced by CO.

Materials And Methods

Soil characteristics and seedling cultivation

Uncontaminated soil (Gleysol) was collected in Ejido Blasillo 4th Section, Huimanguillo, in the state of Tabasco (18° 05' 08.4" N and -93° 56' 50" W). The soil was collected in May, 2020 from a single point on the surface horizon (0–30 cm), was dried, sieved (5 mm mesh), and analyzed to determine its properties before being mixed with different concentrations of fresh oil for the experimental bioassay. The properties of uncontaminated soil were as follows: Sandy clay loam texture (hydrometer method, Bouyoucos, 1962); moderately acid pH (5.5); very high content of organic matter (17.2%) (Walkley and Black, 1934); high content total nitrogen (0.66%) (micro Kjeldhal, Page et al., 1982), high content of sulfate (45 mg/kg) (Etchevers, 1992); and high content phosphate (85 mg/kg) (Olsen and Sommers, 1982). The cultivation of Lh grass seedling was similar to the procedure used by Orocio-Carrillo et al. (2019). Plants of 19.5 ± 2.3 cm in height were produced 30 days after planting; were used for the experimental bioassay.

Soil contamination and experimental design

CO used was medium crude petroleum with an API of 10.8°, specific gravity of 0.84 g/cm; 56.4, 23.7 and 14% of aliphatic, aromatic and polar (resin and asphaltene) fractions, respectively. The CO was obtained from the oil field “Cinco Presidentes” in La Venta, Tabasco, Mexico (18° 12' 11.8" N and -94° 08' 37.8" W) (Orocio-Carrillo et al. 2019). The experiment was carried out under a completely randomized design and 4x2 factorial arrangement: four concentrations of oil [0 (control), 30, 60 y 90 g/kg dw] and two technology, PH with Lh and natural attenuation NA, native microorganisms. A total of eight treatments with six repetitions were maintained in micro-tunnel for 180 days (August 2020 to January 2021) with average temperature of 29 ± 6 °C, relative humidity between 85 and 96% and humidity at field capacity 32 ± 5%. The parameters evaluated in PH treatments were plant growth (number of YP), production of aerial dry matter (ADM) and root dry matter (RDM); hydrogen peroxide in leaf of YP (H_2O_2L) and root (H_2O_2R), ascorbic acid/ascorbate in leaf (AAL) and root (AAR), total phenols in leaf (TPL) and root (TPR), total flavonoids in leaf (TFVL) and root (TFVR), catalase in leaf (CATL) and root (CATR), and phenylalanine ammonia lyase in leaf (PALL) and root (PALR). The parameters evaluated in PH and NA treatments were microbial respiration (CO_2), population (CFU/g) of A*zospirillum* (AZP), *Azotobacter* (AZT) and *Pseudomonas* (PSE) groups, and total petroleum hydrocarbons (TPH) removal.

Plant matter production

On day 180, the amount of YP/tillers was counted, ADM and RDM were harvested. The dry matter of stems, leaves and roots were dried in an oven at 60 °C for 72 h and weighed on a digital scale with a precision of 0.01 g.

Biochemical Analysis in leave and root

Fresh leaves and roots were stored at -80°C until use. The samples were lyophilized (lyophilizer, Yamato Scientific Co. Ltd., Model D401, Santa Clara, CA, USA). ForH_2O_2 (µmol/g dw) compound determination, 25 mg of lyophilized tissue and 1 mL of trichloroacetic acid 0.1%, and quantified in a UV-Vis spectrophotometer (Spectrophotometer UNICO UV2150, Dayton, NJ, USA) with a wavelength of 390 nm (Velikova et al. 2000). The acid/ascorbate (AA) (mg/g dw) compound was determined with the colorimetric method using 2.6 dichlorophenol, 1 g of lyophilized tissue and 1 mL of metaphosphoric acid (1%) (Hung and Yeng 2002). TP (mg/g dw) was determined using the Folin-Ciocalteu reagent in 0.2 g of lyophilized tissue (Cumplido-Nájera et al. 2019). TFV (mg/g dw) was determined according to the method of Arvouet-Grand et al. (1994), 100 mg of lyophilized tissue extracting with reagent-grade methanol and quantifying with a methanolic solution (2%) of aluminum trichloride. The reading was taken at 415 nm in a UV-Vis spectrophotometer. For antioxidant enzyme compound determination, 100 mg of lyophilized tissue and 20 mg of polyvinylpyrrolidone were weighed. After this, 1.5 mL of phosphate buffer with a pH of 7–7.2 (0.1 M) was added, and the mixture was then subjected to micro-centrifugation at 12,000 rpm for 10 min at 4°C. The super natant was filtered with a nylon membrane. With this extract, antioxidants capacity in hydrophilic compounds were determined. CAT (EC 1.11.1.6) (UTP1, where U is equal to the mM equivalent of H_2O_2 consumed per mL/min) was quantified using the method of Dhindsa et al. (1981). PAL (EC 4.3.1.5) was determined according to the method of Sykłowska-Baranek et al. (2015).

Population of rhizobacteria

The effects of the four oil levels and the two soil remediation options were determined by counting viable cells from four groups of microorganisms established in specific culture media (Gonzalez-Moscoso et al. 2019). AZP bacteria were seeded in congo rojo agar culture medium (Döbereiner et al. 1966). AZT bacteria in Asby agar culture medium (Döbereiner et al. 1966), and PSE bacteria on cetrimide + glycerol agar culture medium (Garrity et al. 2005). The cultures were incubated at 28°C, and incubation was for 72 h. The colonies of bacteria was counted and expressed as colony forming units (CFU) per gram of soil. Microbial respiration was measured by CO_2 emission after incubation using alkaline solution and titration with the method described by Stotzky (1965).

Analysis of total petroleum hydrocarbons

The effects of the combination of the NA and PH treatment were determined by percentage of elimination of TPH. Simple soil samples were collected on days 1 and 180 after sowing the grass and the oil was extracted for 8 h in soxhlet equipment (USEPA 1996) with analytical grade dichloromethane. The amount of CO was calculated by weight difference between the quantity extracted on day 1 and the quantity corresponding to day 180 and the difference was multiplied by 100 (Gonzalez-Moscoso et al. 2019).

Statistical analysis of data

Unifactorial ANOVA was used to analyze the data of number of YP, ADM and RDM, and also H_2O_2, AA, TF, TFV, CAT and PAL in leaf of the YP and root according to the CO concentration. Bifactorial ANOVA was used to analyze the data of CFU of rhizobacteria (AZS, AZT, and PSE), CO_2 and TPH removal according to technologies and CO concentration. Data homogeneity was verified. The differences between variables were analyzed using Duncan’s separation of means ($p \leq 0.05$). Bivariate Pearson correlation was used to analyze all the variables in the four PH treatments. All statistical analyses were performed using the statistical program SAS v.8.01 (SAS 2005).

Results

Effect of crude oil on the growth of *Leersia hexandra*

Table 1 shows the results of YP, ADM and RDM of Lh. It stands out that incorporating CO to the soil was associated with significant differences between mean values (Duncan $p \leq 0.05$) in YP and RDM but not in ADM (dried stems and leaves). The oil induced a hormetic effect on the number of YP and the production of RDM. Exposure to 90 g of CO promoted an increase of up to 76% in the number of new plants compared to the control soil. The results of RDM increased

up to 66% in the most contaminated soil (90 g/kg of CO) compared to the control soil. Unlike the roots, the aerial dry biomass of Lh decreased up to 44% with the highest dose of CO (Table 1).

Effect of petroleum on the production of hydrogen peroxide and antioxidants

Table 2 shows the contents of CAT, PAL and H_2O_2 in leaf and root of Lh grass exposed to four different doses of CO. The mean concentrations of these three substances in leaf and root showed statistically significant differences ($p \leq 0.05$). The H_2O_2 content in leaf YP increased up to 32% in plants exposed for 180 days to 90 g/kg of CO. The same concentration of CO induced a phytotoxic process in the root and the content of H_2O_2 decreased by 21%, from 0.62 to 0.49 µmol/g (Table 2). The AA in the leaf varied from 2.3 to 2.5 mg and showed a similar variation in the root, from 2.3 to 2.4 mg. All treatments produced similar results in both plant organs (Duncan $p \leq 0.05$) (Table 3). In the case of TP, exposure to CO induced a hormetic response in the leaf of YP subjected to the three oil treatments. The highest concentration was 12 ± 0.86 mg of TP from 90 g/kg of CO, 34% higher compared to the treatment without CO (Table 3). The opposite effect occurred in root, where the same concentrations inhibited 56% of the TP biomarker. Regarding the production of TFV in leaf and root, it increased in contaminated soils, showing statistically significant differences (Table 3). The concentration of TFVL of plants exposed to 90 g of CO was 2181 mg/100 g. The presence of CO increased the amount of TFVL by 26% compared to the control. In root, the concentration of FV was 33% higher (325 mg) in plants exposed to 30 g of CO, compared to the control. The concentration of the antioxidant enzyme CATL and CATR showed statistically significant differences (Duncan $p \leq 0.05$) when the plants were exposed to a CO concentration of 30 g (Table 2). The CAT increased by 17 and 42.7% in leaf and root, respectively. The concentration of the PAL enzyme in the root showed a significant increase in the presence of oil. The three evaluated doses promoted the production of PAL. The highest concentration reached was 3.0 ± 0.38 U/g of protein, 73% more than obtained with the control soil (Table 2). In leaf, the production of PAL was inhibited by the presence of CO.

Oil and rhizobacteria technology

The two technologies, PH and NA, used for the recovery of soils contaminated with up to 90 g/kg of CO showed significant differences in the population of three groups of plant growth-regulating bacteria and in microbial respiration (Table 4). The AZP bacteria were generally more abundant in NA than in PH. In NA, the highest population ($95x10^3$ CFU) was recorded in soil with 30 g of CO, 12% higher than in the control soil. The AZT bacteria reached the highest population with PH in the presence of 60 and 90 g of CO, ranging from 438 to $481x10^3$ CFU, 52 to 56% higher than in the control soil, respectively. The PSE bacteria formed less CFU than the two other bacteria groups, in the order of 10^1 CFU, two units less. The presence of CO inhibited this group of bacteria, both in PH and NA. Microbial respiration increased up to 75% due to the effect of PH, and 67% due to NA (Table 4).

Crude oil removal according to technology

The data of CO removal for PH and NA on day 180 show an inversely proportional trend to the level of soil contamination (Fig. 1). Highest removal was 57.6% in soil with 30 g/kg of CO subjected to NA, on the other hand, the lowest was 22% with PH in soil with 90 g/kg of crude oil.

Discussion

Growth and production

Soil contamination with CO causes abiotic stress in plants (Tariq *et al.* 2020). In plants, stress can be physiological and biochemical, and it can have positive and negative manifestations. The results of the present study indicate a direct relationship between the amount of CO and the amounts of YP and RDM, but the relationship is inverse with respect to ADM (Table 1). These results are different from those reported by Plice (1948) and Adieze et al. (2012) in *Trifolium* sp, *Centrosema* sp and *Panicum maximum* plants, whose growth increased with low doses of CO of 1%, but decreased with higher doses. Other authors differ, stating that CO does not inhibit plant growth. On the contrary, they mention that low and high doses (7.9 to 238 g/kg CO) promote the production of RDM and YP in *Lh* grass (Alejandro-Córdova et al. 2017; Orocio-Carrillo et al. 2019), which is evidenced by a J-shaped hormetic stress response for both parameters. This positive response to stress in Lh can be explained by a natural evolutionary adaptation to stress conditions (Agathokleous et al. 2020). Rivera-Cruz et al. (2016) found that Lh can remain for up to three years in soils contaminated with 60 to 119 g/kg of TPH, self-regulating its growth even when used to feed cattle. It is known that grasses survive and tolerate CO hydrocarbons in the soil, due to their fasciculated root system with abundant fine roots attached to rhizomes emerging from stem stolons in contact with the soil (Merkl et al. 2005; Rivera-Cruz et al. 2016). Rhizomes extending over the surface horizon in tropical wetlands form an extensive root system together with plant tillers (Rodríguez-Rodríguez et al. 2016). The abundant root growth in Lh grass under stress conditions shows the existence of mechanisms that effectively regulate the concentration of hormones and H_2O_2 as chemical signals that regulate cell activities (Segura 2008; Quan et al. 2008). However, although grasses regulate CO-induced stress, cell death by physical contact of CO with the roots of Lh does not have a negative effect on the plant, in contrast with what has been reported in other plants in which the oil clogs root pores and disrupts water osmosis (Sangeetha and Thangadurai 2014) and the absorption of nutrients (Osuagwu et al. 2013; Tahseen et al. 2016). The resistance of Lh roots to oil-induced stress may be explained by the metabolic induction of antioxidant production and premature lignification, which thickens the root wall (Vanholme et al. 2012). Root thickening in the presence of CO was observed in Lh roots in this study (unpublished data).

The stimulatory effect of CO on the multiplication and growth of YP from the base of the stem and root system of Lh grass may be the result of the microbial degradation of hydrocarbons (Maier and Gentry 2015) and the consequent increase of nitrogen, phosphorus, potassium and sulfate in the soil (Rivera-Cruz et al. 2012; Trujillo-Narcía et al. 2018). The Gleysol soil used in this research was very fertile, containing high levels of total N (0.20%) and extremely rich in PO_4^{-2} (82 mg/kg) and SO_4^{-2} (39 mg/kg). According to Clarkson et al. (2000), these levels of available soil nutrients promote stomatal conductance and hydraulic conductivity in roots under stress conditions. There are claims that plants increase their growth in soil contaminated with CO because “petroleum auxins”, identified as naphthenic acids, improve crop yields, stimulate photosynthesis and increase protein nitrogen (Baker 1970).

H_2O_2 and antioxidants in *Leersia hexandra* leaf

The study of oxidative stress and the antioxidant response induced by hydrocarbons and inorganic elements of CO in plants is a complex affair. The effects of CO contamination on living beings have been studied mainly by considering how CO affects the soil. CO causes changes in temperature, hydrogen potential, electrical conductivity, water availability, and nutrient availability. It also favors the accumulation of heavy metals and toxic hydrocarbon metabolites derived from degradation and mineralization (Devatha et al. 2019; Odukoya et al. 2019). The results of the present study indicate a high concentration of H_2O_2L in YP in the presence of CO and a highly significant positive relationship between them (0.791**, Table 5). The increase in H_2O_2 is in agreement with the results of Burritt (2008), Teotia and Singh (2014), and Ahmad et al. (2020), but there is no agreement on the H_2O_2L*YP relationship. In the Krona variety of rye (*Secale cereale*) H_2O_2 increases with 12% of oil, but RDM and ADM decrease (Skrypnik et al. 2021). Ahmad et al. (2020) reported that 5 and 10% concentrations of hydrocarbons inhibit the production of leaf biomass in *Brassica oleracea*, but increases the concentration of H_2O_2 in leaf. Gutiérrez-Martínez et al. (2020) reported an increase of 16% in the concentration of H_2O_2 in the leaves of *Phaseolus vulgaris* and a decrease in the leaf biomass exposed to 1 µM of Cd.

The excessive accumulation of H_2O_2 in Lh leaf is thus a biomarker of oxidative stress. It is evidence that the plant metabolism regulates physiological processes of senescence by eliminating the production of H_2O_2 at the right place and time (Foyer and Noctor 2003; Bhattacharjee 2010; Hasanuzzaman et al. 2014) since high levels of ROS have a deleterious effect on plasma membranes (Esim and Atici 2015; Kaya et al. 2019). The positive relationship (0.812** and 0.678**, Table 5) between the concentrations of TPL and TFVL and the concentration of CO indicates that these substances can serve as stress biomarkers at high concentrations of CO in the soil. However, CATL increases only at low doses of CO (Table 2). TPL and TFVL contribute to stress resistance and interact with many enzymes, growth regulators, and antioxidants in the plant, although the latter is still under debate (Treutter 2010). These compounds are also mediators of ROS homeostasis in vacuoles, acting mainly as antioxidants (Agati and Tattini 2010). The results of the present study indicate an increase in TP in Lh leaf due to the effect of CO. Similar results were reported in Krona and Valdai rye with 12% of CO (Skrypnik et al. 2021) and in *C. leucanthemum* with 7.5% CO (Noori et al, 2012). TFVL also increase in the presence of CO, similar to the behavior of phenanthrene in *Arabidopsis thaliana* (Lukaszewicz et al. 2004; Fini et al. 2011) and *Chrysanthemum leucanthemum* with 7.5 to 10% of CO (Noori et al. 2012). The regulation of redox homeostasis in plants under polluted conditions is based on the activity of low molecular weight molecules of the enzymatic and non-enzymatic antioxidant systems (Skrypnik et al. 2021). Some authors assume that the enzymatic system provides is the metabolic process that provides the most effective protection against damage caused by ROS (Quan et al. 2008). In the present study, the positive correlation of TPL (0.575*) and TFVL (0.718**) with H_2O_2L (Table 5) confirmed that the enzymatic system may serve as a self-regulatory mechanism for the removal of H_2O_2 in YPL. However, Hernández et al. (2009) and Agati et al. (2012) indicate that the correlation between oxidative stress, ROS generation, and ROS removal activity by TP and TFV remains a controversial subject because the interaction between antioxidant enzymes, substrate (flavonoid), and H_2O_2 occurs only after the rupture of the tonoplast membrane. In the present study, the highly significant positive relationship between YP and TPL (0.845**) and TFVL (0.706**) (Table 5) suggests that these non-enzymatic antioxidants are stress signalers that contribute to YP growth. These compounds cause changes in the levels of 3-indoleacetic acid and the biosynthesis of LEA (late embryogenic abundant) proteins that bind to cell membranes and proteins of cell reproductive organelles (Matilla 2008).

In YPL, CAT was more sensitive than TP and TFV. The production of this enzyme was stimulated only at low doses of CO, showing a negative relationship with CO (-0.530*, Table 5). These results are similar to those reported by Al-Hawas et al. (2012) in *Simmondsia chinensis*. In that case, the concentration of CATL increased in plants exposed to 1% CO, but decreased when exposed to doses of 2 and 3%. In *A. thaliana*, CAT behaves in a similar way when the plant is exposed to phenanthrene; in *Kandelia candle*, when exposed to any of the 16 polycyclic aromatic hydrocarbons (Song et al. 2011; Liu et al. 2014); in *Lolium perenne* to phenol-polycyclic aromatic hydrocarbons (Malincka et al. 2021); and in *Cynara cardunculus* var altilis, when exposed to 100mM NaCl (Docimo et al. 2020). The inhibition of CAT in YPL exposed to CO indicates that the presence of oil disrupts cellular synthesis, which directly induces the accumulation of H_2O_2 in leaf, considering that this antioxidant is directly involved in converting H_2O_2 into water and oxygen under stressful conditions that cause ROS to accumulate (Glorieux and Calderón 2017).

H_2O_2 and antioxidants in *Leersia hexandra* root

Plant roots adapt to different soil conditions (López-Bucio et al. 2003). In the roots of Lh, the amount of synthesized H_2O_2 decreases according to the concentration of CO (-0.937**, Table 5). Similar behavior is observed in the root of *Glycine max* exposed to NaCl (Neves et al. 2010). An opposite behavior is observed in the roots of *G. max* exposed to Cd (Finger-Teixeira et al. 2010) and in *Rumex dentatus*, *Euphorbia helioscopia*, *Cannabis sativa* and *Parthenium hysterophorus* exposed to Pb and Cr (Ullah et al. 2019). These recent findings show there is variation in the synthesis of H_2O_2 in the root of different plants when exposed to stress. Each type of plant regulates the synthesis of H_2O_2 differently. H_2O_2 is a signal molecule involved in acclimation signaling that triggers stress tolerance (Gill and Tuteja 2010). The low concentration of H_2O_2 in the root of Lh exposed to CO suggests that it is a stress signaling molecule, but this concentration is sufficient for promoting plant growth and for preserving the permeability of the cellular channels that conduct calcium and potassium for cellular homeostasis (Foreman et al. 2003; Kwak 2003), cell cycle regulation (Mittler et al. 2004), growth and development (Foreman et al. 2003) and the timely translation of genetic signals (Quan et al. 2008).

The synthesis of root antioxidants as stress biomarkers behaved differently than YPL. In the roots, CO negatively affected TPR (-0.876**) and positively PALR (0.864**). The behavior of TPR whenLh is subjected to oil-induced stress is similar to that of *Lens culinaris* cv. Tub exposed to 0.5 mM Cu^{2+} (Janas et al. 2009). The opposite behavior is observed in *C. leucanthemum* exposed to 10% of CO (Noori et al. 2012). The significant negative relationship TPR*RDM (-0.897**, Table 5) suggests that TP possibly promotes increased root growth by contributing to control the endogenous levels of 3-indoleacetic acid that the plant requires for its growth (Engels et al. 2012). In the present study, Lh regulates the physiological balance of the roots under stress based on a significant and positive relationship TPR*H_2O_2 (0.817**, Table 5). The CAT in the root shows no relationship with H_2O_2R and RDM, but increased in roots exposed to 30, 60 and 90 g/kg of oil, compared to the control. This behavior is different than that observed in the roots of *Lactuca sativa*, *Cichorium endivia*, *Apium*

graveolens, *Petroselinum crispum*, and *Solanum melongena*, but similar to the behavior observed in *Lycopersicon esculentum* exposed to 3-amino-1,2,4-triazole (Chioti and Zervoudakis 2017). Since CAT has been reported to be synthesized in the mitochondria (Willekens et al. 1995) and H_2O_2 is generated during mitochondria respiration (Giorgio et al. 2007), the physiological role of this isoenzyme in the roots of Lh could be to promote the reduction of H_2O_2 by oxidizing it and obtaining water and oxygen, as indicated by Glorieux and Calderon (2017). An outstanding result of the present study is the increase in the concentration of PAL in the roots of Lh. A highly significant positive relationship of this enzyme with CO (0.864**) and RDM (0.709**, Table 5) indicates the protective role it plays in the roots and as a stress biomarker. Similar behavior has been observed in the roots of *G. max* exposed to Cd (Finger-Teixeira et al. 2010). An opposite behavior has been observed in *G. max* and *Z. mays* exposed to dopamine (Guidotti et al. 2013; Siqueira-Soares et al. 2013). In *G. max* and *Beta vulgaris* treated with mannan oligosaccharide for the control of *Meloidogyne javanica* nematodes, where PAL is an indicator of resistance to stress (Débia et al. 2021). PAL biosynthesis has been reported to increase in *Pea* seedlings when ethylene was applied to the plants (Hyodo and Yang 1971), which suggests that ethylene is present in soil derived from the degradation of linear petroleum hydrocarbons (Fessenden and Fessenden 1983), which in contact with the roots of Lh induce root growth. PAL synthesis is important for plant defense and growth due to its role in the synthesis of the secondary metabolite phenylpropanoid, which plays a crucial role in the biosynthesis of monolignols, salicylic acid, phytoalexin, and flavonoids (Deng and Lu 2017; Odukoya et al. 2019).

Bacteria and removal of petroleum hydrocarbons

Various studies have identified that ~~the~~ rhizosphere of grasses used to remove TPH in soils contaminated with CO host an intense microbial activity, including bacteria that regulate plant growth (Arias-Trinidad et al. 2017; Rodríguez-Rodríguez et al. 2016). The increase in the population of *Azotobacter* spp (0.850**, Tables 2 and 5) in the rhizosphere of Lh growing in soil contaminated with CO is similar to the results reported in other studies of Lhexposed to 60-180 g/kg of TPH (Arias-Trinidad et al. 2017) and *Paspalum virgatum* exposed to 25 g/kg of TPH (Rivera-Cruz 2011). The increase in the population of *Azotobacter* spp in the rhizosphere of Lh in the presence of CO is a consequence of the ability of these bacteria to adapt to and resist stress conditions by forming cysts in response to changes in organic substances such as ethanol, n-butan-1-ol or β hydroxybutyrate (Aasfar et al. 2021). The adaptative capacity of *Azotobacter* spp explains its positive relationship with TPH removal (0.597*, Table 5). In contrast, *Azospirillum* spp and *Pseudomonas* spp showed no relationship with TPH removal, and their populations were higher in soil with NA. Under CO-induced stress conditions, *Azotobacter* may acquire plasmid-encoded catabolic genes through horizontal gene transfer processes (Maier and Gentry 2015). Sun et al. (2012) and Penton et al. (2013) mention that stress-adapted bacteria promote the interaction of proteins and DNA with polycyclic aromatic hydrocarbons such as naphthalene, fluorene and pyrene, which oxidize them and form metabolites that are incorporated into the catabolic pathways of the cell, releasing energy. The Lh rhizosphere induces the removal of 22 to 37% of TPH in soil containing between 30 and 90 g/kg of CO. The removal of TPH induced by PH has a positive relationship with RDM (0.601**) and the population of *Azotobacter* spp (0.592*). However, NA achieved a higher removal percentage of between 30 and 57.6%. The removal of TPH by PH from the grasses has been reported by various authors (Liao et al. 2015; González-Moscoso et al. 2019), who recognize that it is a long-term technology with the advantage of providing environmental services to living organisms. Other experimental results (González-Moscoso et al. 2019; Orocio-Carrillo et al. 2019) show thatLh can remove up to 76.6% TPH within six to eight months, but NA yields better results. These authors explain that the higher percentage of removal in non-rhizosphere soil may be because in the soil, the immediate source of organic carbon is petroleum hydrocarbons, while in the rhizosphere the microorganisms have two sources of carbon: root exudates (amino acids, auxins, ethylene, flavonoids, organic acids, palmitic acid, quercetin, strigolactone, ~~and~~ sugars exuded by the root) (Kuzmicheva et al. 2017), and petroleum hydrocarbons, which leads to a process of cometabolism in which microorganisms use both carbon sources. Furthermore, the colonization of the grass rhizosphere by *Azotobacter* and the inhibition of *Azospirillum* and *Pseudomonas* affect the production of root exudates, which can vary depending on the exposure to stress and other factors (Philippot et al. 2013; Pérez-Jaramillo et al. 2015). One of these factors is the amount of detached root cells, which are labile and easily degraded, that incorporate into the soil (Neumann and Römheld 2012). The roots of Lh have a high content of TFV and PAL (Table 2) and, according to Badri et al. (2013), these natural phytochemicals in the rhizosphere stimulate and inhibit a wide variety of microbial communities. In this regard, this study shows a significant positive relationship between AZT and TPR (0.868**) and with PALR (0.622**, Table 5).

Conclusions

The presence of CO in the soil promotes the growth of YP, the production of RDM, H_2O_2, TP and TFV in leaves. The production of CAT and PAL enzymes also increases in Lh roots, but H_2O_2 and TP decrease in roots, even though root production increases. The root system regulates stress by synthesizing low concentrations of H_2O_2 but high concentrations of the enzymes CAT and PAL, maintaining increased RDM production. The increased growth of roots in soil with oil provides the Lh grass with more nutrients and induces an increase in the production of YP or tillers. The roots of grass downregulate the synthesis of H_2O_2 to avoid cell damage in balance with the production of non-enzymatic antioxidants, TP and TFV in the leaf. The root system of Lh associated with AZT spp induces the removal of 37, 24 and 22% of HTP in soil with 30, 60 and 90 g of oil, respectively. Lh is proposed for use as a remediation technology in soil contaminated with up to 90 g/k oil due to its capacity to adapt to stress conditions, manifested by regulating the synthesis of H_2O_2 and antioxidants in the leaf and root during the removal of THP in clay soil in the Mexican humid tropics.

Declarations

Acknowledgments

José Alberto Orocio-Carrillo thanks the Consejo Nacional de Ciencia y Tecnología (CONACYT) of Mexico for the scholarship no. 813712 granted during his Philosopher Doctor studies.

Author contribution

Conceptualization: MCRC. Data curation: MCRC, AJM. Formal analysis: MCRC, AJM, YGG. Funding acquisition: MCRC. AJM. Investigation: MCRC, JAOC, YGG, CCBM. Methodology: MCRC, AJM, YGG, CCBM. Project administration and resource: MCRC, AJM. Software: MCRC, ATN. Supervision and validation: MCRC, AJM. Writing-original draft: MCRC, JAOC. Writing - review and editing: MCRC, ATN. Financing provided by the College of Postgraduates in Agricultural Sciences Campus Tabasco, through the global project "Soil, microorganisms and plants in the sustainability of the environment impacted by pollution."

Data availability Not applicable.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Aasfar A, Bargaz A, Yaakoubi K, Hilali A, Bennis I, Zeroual Y, Meftah Kadmiri I (2021) Nitrogen Fixing Azotobacter Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. Front Microbiol 12:628379. https://doi.org/10.3389/fmicb.2021.628379
2. Adieze EI, Orji CJ, Nwabueze RN, Onyeze GOC (2012) Hydrocarbon stress response of four tropical plants in weathered crude oil contaminated soil in microcosms. Int J Environ Stud 69(3):490–500. https://doi.org/10.1080/00207233.2012.665785
3. Afzal M, Rehman K, Shabir G, Tahseen R, Ijaz A, Hashmat AJ, Brix H (2019) Large-scale remediation of oil-contaminated water using floating treatment wetlands. npj Clean Water 2(3):1–10. https://doi.org/10.1038/s41545-018-0025-7
4. Agathokleous E, Barceló D, Tsatsaki A, Calabrese JE (2020) Hydrocarbon-induced hormesis: 101 years of evidence at the margin? Environ Pollut 265 Part B 114846. https://doi.org/10.1016/j.envpol.2020.114846
5. Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: Location and functional significance. Plant Sci 196:67–76. http://dx.doi.org/10.1016/j.plantsci.2012.07.014
6. Agati G, Tattini M (2010) Multiple functional roles of flavonoids in photoprotection. New Phytol 186(4):786–793. https://doi.org/10.1111/j.1469-8137.2010.03269.x
7. Agathokleous E, Calabrese EJ (2020) Environmental toxicology and ecotoxicology: How clean is clean? Rethinking dose-response analysis. Sci Total Environ 746:138769. https://doi.org/10.1016/j.scitotenv.2020.138769
8. Ahmad A, Khan UW, Shah AA, Ahmad YN, Ali A, Rizwan M, Ali S (2020) Dopamine Alleviates Hydrocarbon Stress in Brassica oleracea through Modulation of Physio-Biochemical Attributes and Antioxidant Defense Systems. Chemosphere 270:128633. https://doi.org/10.1016/j.chemosphere.2020.128633
9. Alanbary SRN, Abdullah SRS, Al-Baldawi IAW, Hasan HA, Anuar N, Othman AR, Suja' F (2019) Phytotoxicity of Contaminated Sand Containing Crude Oil Sludge on Ludwigia octovalvis. J Ecol Eng 20(11):246–255. https://doi.org/10.12911/22998993/113189
10. Al-Baldawi IA, Abdullah SRS, Anuar N, Suja F, Mushrifah I (2015) Phytodegradation of total petroleum hydrocarbon (TPH) in diesel-contaminated water using Scirpus grossus. Ecol Eng 74:463–473. https://doi.org/10.1016/j.ecoleng.2014.11.007
11. Alejandro-Córdova A, Rivera-Cruz MC, Hernández-Cuevas LV, Alarcón A, Trujillo-Narcía A (2017) Responses of Arbuscular Mycorrhizal Fungi and Grass Leersia hexandra Swartz Exposed to Soil with Crude Oil. Water Air Soil Pollut 228:65. https://doi.org/10.1007/s11270-017-3247-2
12. Al-Hawas GHS, Shukry WM, Azzoz MM, Al-Moaik RMS (2012) The effect of sublethal concentrations of crude oil on the metabolism of Jojoba (Simmodsia chinensis) seedlings. Int Res J Plant Sci 3(4):54–62
13. Al-Mansoory AF, Idris M, Abdullah SRS, Anaur N (2017) Phytoremediation of contaminated soils containing gasoline using Ludwigia octovalvis (Jacq.) in greenhouse pots. Environ Sci Pollut Res 24(13):11998–12008. https://doi.org/10.1007/s11356-015-5261-5
14. Arias-Trinidad A, Rivera-Cruz MC, Roldán-Garrigós A, Aceves-Navarro LA, Quintero-Lizaola R, Hernández-Guzmán J (2017) Uso de Leersia hexandra (Poaceae) en la fitorremediación de suelos contaminados con petróleo fresco e intemperizado. Rev Biol Trop 65(1):21–30
15. Arvouet-Grand A, Vennat B, Pourrat A, Legret P (1994) Standardization of propolis extract and identification of principal constituents. J Pharm Belg 49(6):462–468
16. Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolicrelated compounds predominantly modulate the soil microbiome. J Biol Chem 288(7):4502–4512. https://doi.org/10.1074/jbc.M112.433300
17. Baker J (1970) The efects of oils on plants. Environ Pollut 1(1):27–44. https://doi.org/10.1016/0013-9327(70)90004-2
18. Bhattacharjee S (2010) Sites of generation and physicochemical basis of formation of reactive oxygen species in plant cell. In: Gupta SD (ed) Reactive Oxygen Species and Antioxidants in Higher Plants. CRC Press, New York, USA, pp 1–30. https://doi.org/10.1201/9781439854082
19. Bouyoucos GJ (1962) Hydrometer method improved for making particle size analyses of soils. Agron J 54(5):464–465. https://doi.org/10.2134/agronj1962.00021962005400050028x
20. Burritt JD (2008) The polycyclic aromatic hydrocarbon phenanthrene causes oxidative stress and alters polyamine metabolism in the aquatic liverwort Riccia fluitans L. Plant Cell Environ 31:1416–1431. https://doi.org/10.1111/j.1365-3040.2008.01846.x
21. Chen F, Reddy MSS, Temple S, Jackson L, Shadle G, Dixon RA (2006) Multi-site genetic modulation of monolignol biosynthesis suggests new routes for formation of syringyl lignin and wall-bound ferulic acid in alfalfa (Medicago sativa L.). Plant J 48(1):113–124. https://doi.org/10.1111/j.1365-313X.2006.02857.x

22. Chioti V, Zervoudakis G (2017) Is Root Catalase a Bifunctional Catalase-Peroxidase? Antioxidants 6(39):1–6. https://doi.org/10.3390/antiox6020039
23. Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E (2000) Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. J Exp Bot 51(342):61–70. https://doi.org/10.1093/jexbot/51.342.61
24. Cumplido-Nájera CF, González-Morales S, Ortega-Ortíz H, Cadenas-Pliego G, Benavides-Mendoza A, Juárez-Maldonado A (2019) The application of copper nanoparticles and potassium silicate stimulate the tolerance to Clavibacter michiganensis in tomato plants. Hortic Sci 245:82–89. https://doi.org/10.1016/j.scienta.2018.10.007
25. Dat J, Vandenabeele S, Vranova E, van Montagu M, Inze D, van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795. https://doi.org/10.1007/s000180050041
26. del Río LA, Sandalio LM, Corpas FJ, Palma JM, Barroso JB (2006) Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol 141(4):330–335. https://doi.org/10.1104/pp.106.078204
27. Débia GDJ, Barros BCB, Puerari HH, Ferreira JCA, Dias-Arieira CR (2021) Induced systemic resistance in beet plants infected with Meloidogyne javanica. Chil J Agric Anim Sci 81(1):70–79. http://dx.doi.org/10.4067/S0718-58392021000100070
28. Deng Y, Lu S (2017) Biosynthesis and regulation of phenylpropanoids in plants. Crit Rev Plant Sci 36(4):257–290. https://doi.org/10.1080/07352689.2017.1402852
29. Devatha CP, Vishal AV, Chandra RPJ (2019) Investigation of physical and chemical characteristics on soil due to crude oil contamination and its remediation. Appl Water Sci 9:89. https://doi.org/10.1007/s13201-019-0970-4
30. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decrease levels of superoxide dismutase and catalase. J Exp Bot 32(1):93–101. https://doi.org/10.1093/jxb/32.1.93
31. Döbereiner J, Marriel IE, Nery M (1966) Ecological distribution of Spirillum lipoferum Beijerinck. Can J Microbiol 22(10):1464–1473. https://doi.org/10.1139/m76-217
32. Docimo T, De Stefano R, Cappetta E, Piccinelli AL, Celano R, De Palma M, Tucci M (2020) Physiological, Biochemical, and Metabolic Responses to Short and Prolonged Saline Stress in Two Cultivated Cardoon Genotypes. Plants 9(5):1–18. https://doi.org/10.3390/plants9050554
33. Engels C, Kirkby E, White P (2012) Mineral Nutrition, Yield and Source-Sink Relationships. Marschener P (ed). 3er. Academic Press Elsevier. San Diego CA, USA, pp 85–133. https://doi.org/10.1016/B978-0-12-384905-2.00005-4
34. Esim N, Atici EO (2015) Effects of exogenous nitric oxide and salicylic acid on chilling-induced oxidative stress in wheat (Triticum aestivum). Front Life Sci 8(2):124–130. https://doi.org/10.1080/21553769.2014.998299
35. Etchevers BJD (1992) Manual de métodos para análisis de suelos, plantas agua y fertilizantes. Análisis rutinarios en estudios y programas de fertilidad. Laboratorio de Fertilidad, Centro de Edafología. Colegio de Postgraduados en Ciencias Agrícolas. Montecillos. edo, México
36. Fan D, Wang S, Guo Y, Liu J, Agathokleous E, Zhu Y, Han J (2021) The role of bacterial communities in shaping Cd-induced hormesis in 'living' soil as a function of land-use change. J Hazard Mater 409:124996. https://doi.org/10.1016/j.jhazmat.2020.124996
37. Fessenden RJ, Feseenden JS (1983) Química orgánica. Grupo Editorial Iberoamérica. México D.F. 1076 p
38. Fini A, Brunetti C, Di Ferdinando M, Ferrini F, Tattini M (2011) Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. Plant Signal Behav 6(5):709–711. https://doi.org/10.4161/psb.6.5.15069
39. Finger-Teixeira A, Ferrarese LML, Soares AR, da Silva D, Ferrarese-Filho O (2010) Cadmium-induced lignification restricts soybean root growth. Ecotox Environ Safe 73(8):1959–1964. https://doi.org/10.1016/j.ecoenv.2010.08.021
40. Foreman J, Demidchik V, Bothwell HJ, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones DJ, Davies MJ, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422:442–446. https://doi.org/10.1038/nature01485
41. Foyer CH, Noctor G (2003) Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119(3):355–364. https://doi.org/10.1034/j.1399-3054.2003.00223.x
42. Fukao T, Bailey-Serres J (2004) Plant responses to hypoxia-is survival a balancing act? Trends Plant Sci 9(9):449–456. https://doi.org/10.1016/j.tplants.2004.07.005
43. Garrity GM, Bell JA, Lilburn T (2005) Order IX. Pseudomonadales Orla-Jensen 1921, 270AL. In: Brenner DJ, Krieg NR, Staley JT (eds) Bergey's Manual® of Systematic Bacteriology. Springer, Boston, MA, pp 323–442. https://doi.org/10.1007/0-387-28022-7_9
44. Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48(12):909–930. https://doi.org/10.1016/j.plaphy.2010.08.016
45. Giorgio M, Trinei M, Migliaccio E, Pelicci PG (2007) Hydrogen peroxide: A metabolic by-product or a common mediator of ageing signals? Nat Rev Mol Cell Biol 8(9):722–728. https://doi.org/10.1038/nrm2240
46. Glorieux C, Calderon PB (2017) Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. Biol Chem 398(10):1095–1108. https://doi.org/10.1515/hsz-2017-0131
47. González-Moscoso M, Rivera-Cruz MC, Delgadillo-Martínez J, Lagunes-Espinoza LC (2017) Analysis of plant growth and production of Leersia hexandra Swartz in the Mexican humid tropics as a function of oil and surfactant
48. Polibotánica 3:177–196. http://dx.doi.org/10.18387/polibotanica.43.8 (in Spanish)
49. González-Moscoso M, Rivera-Cruz MC, Trujillo-Narcía A (2019) Decontamination of soil containing oil by natural attenuation, phytoremediation and chemical desorption. Int J Phytoremediat 21(8):768–776. https://doi.org/10.1080/15226514.2019.1566879

50. Guidotti BB, Gomes BR, Cassia de Siqueiria-Soares R, Soares AR, Ferrares-Filho O (2013) The effects of dopamine on root growth and enzyme activity in soybean seedlings. Plant Signal Behav 8(9):e25477. http://dx.doi.org/10.4161/psb.25477
51. Gutiérrez-Martínez PB, Torres-Moran MI, Romero-Puertas MC, Casas-Sólis J, Zarazua-Villaseñor P, Sandoval-Pinto E, Ramírez-Hernández BC (2020) Assessment of antioxidant enzymes in leaves and roots of Phaseolus vulgaris plants under cadmium stress.Rev Cienc Biol Salud XXII(2):110–118
52. Halliwell B (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 141(2):312–322. https://doi.org/10.1104/pp.106.077073
53. Hasanuzzaman M, Alam MMd, Rahman A, Hasanuzzaman MD, Nahar K, Fijita M (2014) Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. BioMed Res Int 214:1–17. https://doi.org/10.1155/2014/757219
54. Hernández I, Alegre L, van Breusegem F, Munné-Bosch S (2009) How relevant are flavonoids as antioxidants in plants. Trends Plant Sci 14(3):125–132. https://doi.org/10.1016/j.tplants.2008.12.003
55. Hung CY, Yen GC (2002) Antioxidant activity of phenolic compounds isolated from Mesona procumbens Hemsl. J Agric Food Chem 50(10):2993–2997. https://doi.org/10.1021/jf011454y
56. Hyodo H, Yang SF (1971) Ethylene-enhanced Synthesis of Phenylalanine Ammonia-Lyase in Pea Seedlings. Plant Physiol 47(6):765–770. https://doi.org/10.1104/pp.47.6.765
57. Janas MK, Amarowicz R, Zielińska-Tomaszewska J, Kosińska A, Posmyk MM (2009) Induction of phenolic compounds in two dark-grown lentil cultivars with different tolerance to copper ions. Acta Physiol Plant 31:587–595. https://doi.org/10.1007/s11738-008-0268-x
58. Kaya C, Okant M, Ugurlar F, Alyemeni MN, Ashraf M, Ahmad P (2019) Melatonin-mediated nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants. Chemosphere 225:627–638. https://doi.org/10.1016/j.chemosphere.2019.03.026
59. Kuzmicheva YV, Shaposhnikov AI, Petrova SN, Makarova NM, Tychinskaya IL, Puhalsky JV, Parahin NV, Tikhonovich IA, Belimov AA (2017) Variety specific relationships between effects of rhizobacteria on root exudation, growth and nutrient uptake of soybean. Plant Soil 419:83–96. https://doi.org/10.1007/s11104-017-3320-z
60. Kwak JM (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J 22(11):2623–2633. https://doi.org/10.1093/emboj/cdg277
61. Laloi C, Apel K, Danon A (2004) Reactive oxygen signaling: the latest news. Curr Opin Plant Biol 7(3):323–328. https://doi.org/10.1016/j.pbi.2004.03.005
62. Liao C, Xu W, Lu G, Liang X, Guo C, Yang C, Dang Z (2015) Accumulation of hydrocarbons by maize (Zea mays L.) in remediation of soils contaminated with crude oil. Int J Phytorem 17(7):693–700. https://doi.org/10.1080/15226514.2014.964840
63. Liu H, Weisman D, Tang L, Tan L, Zhang W, Wang Z, Huang Y, Lin W, Liu X, Colón-Carmona A (2014) Stress signaling in response to polycyclic aromatic hydrocarbon exposure in Arabidopsis thaliana involves a nucleoside diphosphate kinase, NDPK-3. Planta 241:95–107. https://doi.org/10.1007/s00425-014-2161-8
64. Logeshwaran P, Megharaj M, Chadalavada S, Bowman M, Naidu R (2018) Petroleum hydrocarbons (PH) in groundwater aquifers: An overview of environmental fate, toxicity, microbial degradation and risk-based remediation approaches. Environ Sci Technol 10:175–193. https://doi.org/10.1016/j.eti.2018.02.001
65. López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6(3):280–287. https://doi.org/10.1016/S1369-5266(03)00035-9
66. Lukaszewicz M, Matysiak-Kata I, Skala J, Fecka I, Cisowski W, Szopa J (2004) Antioxidant capacity manipulation in transgenic potato tuber by changes in phenolic compounds content. J Agric Food Chem 52(6):1526–1533. https://doi.org/10.1021/jf034482k
67. Maier MR, Gentry JT (2015) Microorganisms and organic pollutants. In: Pepper LI, Gerba PC, Gentry JT (eds) Environmental Microbiology, 3rd edn. Academic Press, San Diego. https://doi.org/10.1016/B978-0-12-394626-3.00017-X
68. Malicka M, Magurno F, Posta K, Chmura D, Piotrowska-Seget Z (2021) Differences in the effects of single and mixed species of AMF on the growth and oxidative stress defense in Lolium perenne exposed to hydrocarbons. Ecotox Environ Safe 217:112252. https://doi.org/10.1016/j.ecoenv.2021.112252
69. Matilla JA (2008) Seed development and germination Fundamentals of Plant Physiology In: Azcón-Bieto J, Talón M (eds) McGraw. Hill, Interamericana. Barcelona. pp. 537–558 (In spanish)
70. Merkl N, Schultze-Kraft R, Infante C (2005) Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. Water Air Soil Poll 165(1):195–209. https://doi.org/10.1007/s11270-005-4979-y
71. Mickelbart MV, Hasegawa PM, Bailey-Serres J (2015) Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat Rev Genet 16:237–251. https://doi.org/10.1038/nrg3901
72. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9(10):490–498. https://doi.org/10.1016/j.tplants.2004.08.009
73. Moubasher HA, Hegazy AK, Mohamed NH, Moustafa YM, Kabiel HF, Hamad AA (2015) Phytoremediation of soils polluted with crude petroleum oil using Bassia scoparia and its associated rhizosphere microorganisms. Int Biodeterior Biodegrad 98:113–120. http://dx.doi.org/10.1016/j.ibiod.2014.11.019
74. Neumann G, Römheld V (2012) Rhizosphere Chemistry in Relation to Plant Nutrition. In: Marschner P (ed) Marschener's Mineral Nutrition of Higher Plants, 3rd edn. Elsevier, San Diego, pp 347–368. https://doi.org/10.1016/B978-0-12-384905-2.00014-5
75. Neves GYS, Marchiosi RM, Ferrarese LL, Siqueira-Soares RC, Ferrarese-Filho O (2010) Root growth inhibition and lignification induced by salt stress in soybean. J Agron Crop Sci 196(6):467–473. http://dx.doi.org/10.1111/j.1439-037X.2010.00432.x

76. Noori A, Zare H, Alaie E (2012) Changes in total phenol and flavonoid contents in Chrysanthemum leucanthemum under crude oil contamination. J Environ Biol 6(12):3057–3064
77. Odukoya J, Lambert R, Sakrabani R (2019) Understanding the impacts of crude oil and its induced abiotic stresses on agrifood production: a review. Horticulturae 5(47):1–27. http://dx.doi.org/10.3390/horticulturae5020047
78. Omari K, Revitt M, Shutes B, Garelick H (2003) Hydrocarbon removal in an experimental gravel bed constructed wetland. Water Sci Technol 48(5):275–281. https://doi.org/10.2166/wst.2003.0333
79. Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeny DR (eds). Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties. 2nd ed. Madison. pp. 403–430
80. Orocio-Carrillo JA, Rivera-Cruz MC, Aranda-Ibañez M, Trujillo-Narcía A, Hernández-Galvez G, Mendoza-López MR (2019) Hormesis under oil-induced stress in Leersia hexandra Sw. used as phytoremediator in clay soils of the Mexican humid tropic. Ecotoxicology 28(9):1063–1074. https://doi.org/10.1007/s10646-019-02106-1
81. Osuagwu AN, Okigbo AU, Ekpo IA, Chukwurah PN, Agbor RB (2013) Effect of crude oil pollution on growth parameters, chlorophyll content and bulbils yield in air potato (Dioscorea bulbifera L). Int J Appl Sci Technol 3(4):37–42
82. Page AL, Miller RH, Keeney DR (1982) Nitrogen total. Page AL, Miller RH, Keeney DR (eds). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed Madison. Pp. 595–629
83. Penton CR, Johnson TA, Quensen JF, Iwai S, Cole JR, Tiedje JM (2013) Functional genes to assess nitrogen cycling and aromatic hydrocarbon degradation: primers and processing matter. Front Microbiol 4:1–17. http://dx.doi.org/10.3389/fmicb.2013.00279
84. Pérez-Jaramillo JE, Mendes R, Raaijmakers JM (2015) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol 90:635–644. https://doi.org/10.1007/s11103-015-0337-7
85. Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799. https://doi.org/10.1038/nrmicro3109
86. Plice MJ (1948) Some effects a crude petroleum on soil. Fertility Soil Sci Soc Proc 13:413–416. https://doi.org/10.2136/sssaj1949.036159950013000C0075x
87. Quan LJ, Zhang B, Shi WW, Li HY (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J Integr Plant Biol 50(1):2–18. https://doi.orp/10.1111/j.1744-7909.2007.00599.x
88. Rivera-Cruz MC (2011) Bacteria and fungi in oil-contaminated soil in Tabasco. In: Gamboa AM, Rojas HR (eds) Microbial genetic resources in the Gulf-Southeast Zone of Mexico. National Subsystem of Microbial Genetic Resources. Morelia, México, pp 77–87. (in spanish).
89. Rivera-Cruz MC, Maldonado-Chávez E, Trujillo-Narcía A (2012) Effects of crude oil on the growth of Brachiaria mutica and Leucaena leucocephala and on soil and plant macronutrients. Trop Subtrop Agroecosystems 15(SUP 2):S30–S39
90. Rivera-Cruz MC, Trujillo-Narcía A, Trujillo-Rivera EA, Arias-Trinidad A, Mendoza- López MR (2016) Natural attenuation of weathered oil using aquatic plants in a farm in southeast Mexico. Int J Phytoremediat 18(9):877–884. https://doi.org/10.1080/15226514.2016.1156632
91. Rodríguez-Rodríguez N, Rivera-Cruz MC, Trujillo-Narcía A, Almaraz-Suarez JJ, Salgado-García S (2016) Spatial distribution of oil and biostimulation through the rhizosphere of Leersia hexandra in degraded soil. Water Air Soil Poll 227:319. https://doi.org/10.1007/s11270-016-3030-9
92. Sachdev S, Ansari SA, Ansari MI, Fujita M, Hasanuzzaman M (2021) Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. Antioxidants 10(2):277. https://doi.org/10.3390/antiox10020277
93. Sangeetha J, Thangadurai D (2014) Effect of biologically treated petroleum sludge on seed germination and seedling growth of Vigna unguiculata (L.) Walp. (Fabaceae). Braz Arch Biol Tehn 57(3):427–433. https://doi.org/10.1590/S1516-89132014005000011
94. SAS (Statiscal Analysis Systems) (2005) "User's guide", versión 9.1.3. SAS Institute, Inc, Cary, NC, p 664
95. Segura J (2008) Cytoquins. In: Azcón-Bieto J, Talón M (eds). Fundamentals of Plant Physiology. McGraw-Hill Interamericana de España, S.A.U. Barcelona. pp. 421–444 (in Spanish)
96. Siqueira-Soares RC, Soares AR, Parizotto AV, Ferrarese MLL, Ferrarese-Filho O (2013) Root growth and enzymes related to the lignification of maize seedlings exposed to the allelochemical L-DOPA. Sci World J 2013:1–6. http://dx.doi.org/10.1155/2013/134237
97. Soares RA, Siqueira-Soares RC, Salvador V, Ferrarese-Filho O (2012) The effects of L-DOPA on root growth, lignification and enzyme activity in soybean seedlings. Acta Physiol Plant 34(5):1811–1817. https://doi.org/10.1007/s11738-012-0979-x
98. Skrypnik L, Maslennikov P, Novikova A, Kozhikin M (2021) Effect of crude oil on growth, oxidative stress and response of antioxidative System of two rye (Secale cereale L.) varieties. Plants 10(1):157. https://doi.org/10.3390/plants10010157
99. Song H, Wang YS, Sun CC, Wu ML, Peng YL, Deng C, Li QP (2011) Effects of polycyclic aromatic hydrocarbons exposure on antioxidant system activities and proline content in Kandelia candel. Oceanol Hydrobiol Stud 40(3):9–18. https://doi.org/10.2478/s13545-011-0024-5
100. Stotzky G (1965) Microbial respiration. Methods of soil analysis. In: Norman AG (ed) Chemical and Microbiological Properties, Part 2. American Society of Agronomy, New York, pp 1550–1572. https://doi.org/10.2134/agronmonogr9.2.c62
101. Sun FL, Wang YS, Sun CC, Peng YL, Deng C (2012) Effects of three different PAHs on nitrogen-fixing bacterial diversity in mangrove sediment. Ecotoxicology 21(6):1651–1660. https://doi.org/10.1007/s10646-012-0946-8
102. Sykłowska-Baranek K, Grech-Baran M, Naliwajski MR (2015) Paclitaxel production and PAL activity in hairy root cultures of Taxus x media var. Hicksii carrying a taxadiene synthase transgene elicited with nitric oxide and methyl jasmonate. Acta Physiol Plant 37:218. https://doi.org/10.1007/s11738-015-1949-x

103. Tahseen R, Afzal M, Iqbal S, Shabir G, Khan QM, Khalid ZM, Banat IM (2016) Rhamnolipids and nutrients boost remediation of crude oil-contaminated soil by enhancing bacterial colonization and metabolic activities. Int Biodeterior Biodegrad 115:192–198. https://doi.org/10.1016/j.ibiod.2016.08.010
104. Tariq M, Shah AA, Yasin NA, Ahmad A, Rizwan M (2020) Enhanced performance of Bacillus megaterium OSR-3 in combination with putrescine ammeliorated hydrocarbon stress in Nicotiana tabacum. Int J Phytoremediat 23(2):119–129. https://doi.org/10.1080/15226514.2020.1801572
105. Teotia S, Singh D (2014) Oxidative stress in plants and its management. In: Gaur R, Sharma P (eds) Approaches to Plant Stress and Their Management. Springer, New Delhi, pp 227–253. https://doi.org/10.1007/978-81-322-1620-9_13
106. Treutter D (2010) Managing Phenol Contents in Crop Plants by Phytochemical Farming and Breeding-Visions and Constraints. Int J Mol Sci 11:807–857. https://doi.org/10.3390/ijms11030807
107. Trujillo-Narcía A, Rivera-Cruz MC, Trujillo-Rivera EA, Garrigos AR (2018) Reintroducing plant coverage in a tropical wetland contaminated with oil and sulfate: Rhizosphere effects on Desulfovibrio populations. Rev Biol Trop 66(2):908–917. https://doi.org/10.15517/rbt.v66i2.33422
108. Ullah R, Hadi F, Ahmad S, Ullah JA, Rongliang Q (2019) Phytoremediation of lead and chromium contaminated soil improves with the endogenous phenolics and proline production in Parthenium, Cannabis, Euphorbia, and Rumex Species. Water Air Soil Pollut 230:40. https://doi.org/10.1007/s11270-019-4089-x
109. USEPA-3540C (1996) Soxhlet extraction organics. SW-846 test methods for evaluating solid waste physical/chemical methods. http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/3540c.pdf [accessed 2012 Jun 20].
110. Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Sci 151(1):59–66. https://doi.org/10.1016/S0168-9452(99)00197-1
111. Yap CK, Tan WS, Wong KW, Ong GH, Cheng WH, Nulit R, Ibrahim MH, Chew W, Berandah EF, Okamura H, Al-Mutairi KA, Al-Shami SA, Sharifinia M, Mustafa M, Leong WJ, You CF (2021) Antioxidant enzyme activities as biomarkers of Cu and Pb stress in Centella asiatica. Stresses 1(4):253–265. https://doi.org/10.3390/stresses1040018
112. Wang M, Garrido-Sanz D, Sansegundo-Lobato P, Redondo-Nieto M, Conlon R, Martin M, Mali R, Liu X, Dowling DN, Rivilla R, Germaine KJ (2021) Soil microbiome structure and function in ecopiles used to remediate petroleum-contaminated soil. Front Environ Sci 9:624070. https://doi.org/10.3389/fenvs.2021.62407
113. Walkley A, Black IA (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37(1):29–38. https://doi.org/10.1097/00010694-193401000-00003
114. Willekens H, Inzé D, van Montagu M, van Camp W (1995) Catalases in plants. Mol Breed 1:207–228. DOI:10.1007/BF02277422
115. Zuzolo D, Guarino C, Tartaglia M, Sciarrillo R (2021) Plant-Soil-Microbiota combination for the removal of total petroleum hydrocarbons (TPH): An in-field experiment. Front Microbiol 11:621581. https://doi.org/10.3389/fmicb.2020.621581

Tables

Table 1. Quantities of young plants, aerial dry matter and root dry matter of *Leerxia hexandra* exposed to crude oil for 180 days.

Crude oil	Young plants		Dry matter			
(g/kg dw)	Quantity	%	Aerial (g)	%	Root (g)	%
0	26 ± 3.2		148 ± 2.7*		27 ± 1.1	
30	51 ± 2	+49	118 ± 10	-20	62 ± 4.4	+24
60	70 ± 10.2	+63	99 ± 18b	-33	74 ± 3.4	+63
90	108 ± 14*	+76	83 ± 14	-44	80 ± 15.4*	+66
No oil	26		148*		27	
Oil	76*	+66	100	-32	72*	+62
Coefficient of variation	7.3		11.27		5.82	

The symbol % (+) represents an increase and % (-) a decrease in young plants and vegetable dry matter with respect to the values of the control treatment (0 g/kg crude oil). *Treatment mean with greater statistical difference (Duncan, $p \leq 0.05$, n = 6).

Table 2. Changes in catalase (CAT), phenylalanine ammonium lyase (PAL) and hydrogen peroxide (H_2O_2) contents in leaf and root of *Leersia hexandra*exposed to crude oil (CO) for 180 days.

CO (g/kg dw)	CAT (U/g protein)	%	PAL (U/g protein)	%	H_2O_2 (µmol/g dw)	%
Leaf						
0	169 ± 21		12 ± 35*		2.97 ± 0.54	
30	182 ± 40*	+17	10 ± 0.57	-16.6	3.8 ± 0.18	+22
60	169 ± 31	0	10.9 ± 1.2	-9	4.3 ± 0.48*	+31
90	118 ± 16	-30	10.3 ± .98	-14	4.4 ± 0.46*	+32
Root						
0	63 ± 10.7		0.8 ± 0.13		0.62 ± 0.03*	
30	110 ± 26*	+42.7	2 ± 0.25	+60	0.57±.02	-8
60	91 ± 13.5	+30.7	2.4 ± 0.21	+66	0.53 ± 0.01	-16
90	73 ± 9.8	+13.7	3 ± 0.38*	+73	0.49 ± 0.009	-21

The symbol % (+) represents an increase and % (-) a decrease in CAT, PAL and H_2O_2, in leaf and root, with respect to the values of the control treatment (0 g/kg crude oil). *Treatment mean with greater statistical difference (Duncan, $p \leq 0.05$, n = 6).

Table 3. Changes in ascorbic acid (AA), total phenols (TP) and total flavonoids (TFV) contents in leaf and root of *Leersia hexandra* exposed to crude oil for 180 days.

Crude oil (g/kg dw)	AA (mg/g dw)	%	TP (mg/g dw)	%	TFV (mg/100g dw)	%
Leaf						
0	2.3 ±.04*		7.9 ± 1.3		1617 ± 62	
30	2.5±.08*	+0.8	8.4 ± 0.34	+6	2044 ± 13	+21
60	2.4±.01*	+0.4	8.9 ± 0.27	+11	1851 ± 86	+13
90	2.4±.03*	+0.4	12 ± 0.86*	+34	2181±23*	+26
Root						
0	2.4 ± 0.02*		1.23 ± 0.04*		236 ± 6.9	
30	2.3 ± 0.01*	-0.4	0.75 ± 0.12	-39	352 ± 15*	+33
60	2.4 ± 0.03*	0	0.71 ± 0.10	-42	239 ± 5.4	+1.3
90	2.4 ± 0.04*	0	0.54 ± 0.06	-56	251 ± 0.22	+5.9

The symbol % (+) represents an increase and % (-) a decrease in AA, TP and TFV, in leaf and root, with respect to the values of the control treatment (0 g/kg crude oil). *Treatment mean with greater statistical difference (Duncan, $p \leq 0.05$, n = 6).

Table 4. Changes in population of *Azospirillum* (AZP), *Azotobacter* (AZT) and *Pseudomonas* (PSE), and microbial respiration (MR) in soil with crude oil (CO) on day 180 after phytoremediation (PH) and natural attenuation (NA)

Technology/ CO (g/kg)	AZP (10^3 CFU/g dw)	%	AZT (10^3 CFU/g dw)	%	PSE (10 CFU/g dw)	%	MR (mg CO_2/kg dw)	%
PH								
0	84*		212		94*		12	
30	67	-20	371	+43	57	-60.6	19	+37
60	5	-94	481*	+56	33	-64.8	38	+68
90	21	-75	438*	+52	73	-22	48*	+75
NA								
0	84		244		314*	-49	9.6	
30	95*	12	328	+32	160	-85	13	+26
60	70	-17	370	+34	46	-75	28	+66
90	41	-51	364	+33	77		29	+67
PH	44B		375A		64B		29A	
NA	72A		326A		149A		20B	

The symbol % (+) represents an increase and % (-) a decrease in the population densities of the three groups of bacteria and microbial respiration with respect to the values of the control treatment (0 g/kg crude oil). *Treatment mean with greater statistical difference (Duncan, p≤0.05, n = 6).

Table 5. Pearson correlation values between crude oil, plant, antioxidants, phytoremediation and bacteria.

	YP	RDM	H_2O_2L	TPL	TFVL	PALL	H_2O_2R	TPR	TFVR	PALR	REM	AZP	AZT	PSE	MR
CO[a]	.970**	.882**	.778**	.812**	.678**	NS	-.937**	-.876**	NS	.864**	NS	-.977**	.850**	NS	.995**
YP		.831**	.791**	.845**	.706**	NS	-.911**	-.848**	NS	.882**	NS	-.977**	.777**	NS	.972**
RDM			.838**	.652**	.768**	NS	-.831**	-.897**	Ns	.709**	.601*	-.809**	.927**	-.608*	.857**
H_2O_2L				.575*	.718**	NS	-.728**	-.732**	NS	.764**	NS	-.724**	.837**	-.575**	.750**
TPL					.625**	NS	-.801**	-.651**	NS	.807**	NS	-.890**	.509*	NS	.843**
TFVL						-.610*	.718**	-.738**	NS	.693**	.579*	-.693**	.589*	NS	.676**
PALL							NS	NS	-.742**	NS	-.578*	NS	NS	NS	NS
H_2O_2R								-.732**	NS	.764**	NS	-.764**	.837**	-.575*	.750**
TPR									NS	-.595*	-.741**	.824**	-.868**	.593*	-.852*
TFVR										NS	.676**	NS	NS	NS	NS
PALR											NS	-.891**	.622*	NS	.878**
REM												NS	.592*	-.609*	NS
AZP													-.738**	NS	-.985*
AZT														-.773**	.802**
PSE															NS

*Significant correlation at the level p≤0.05, **Highly significant correlation at the level p≤0.01, NS: no significance.

[a]CO: crude oil; YP: young plants; RDM: root dry matter; H_2O_2L: hydrogen peroxide in leaf; TPL: total phenols in leaf; TFVL: total flavonoids in leaf; PALL: phenylalanine ammonia lyase in leaf; H_2O_2R: hydrogen peroxide in root; TPR: total phenols in root; TFVR: total flavonoids in leaf; PALR: phenylalanine ammonia lyase in root; REM: removal of crude oil; AZP: *Azospirillum* spp; AZT: *Azotobacter* spp; PSE: *Pseudomonas* spp. MR: Microbial respiration.

Figures

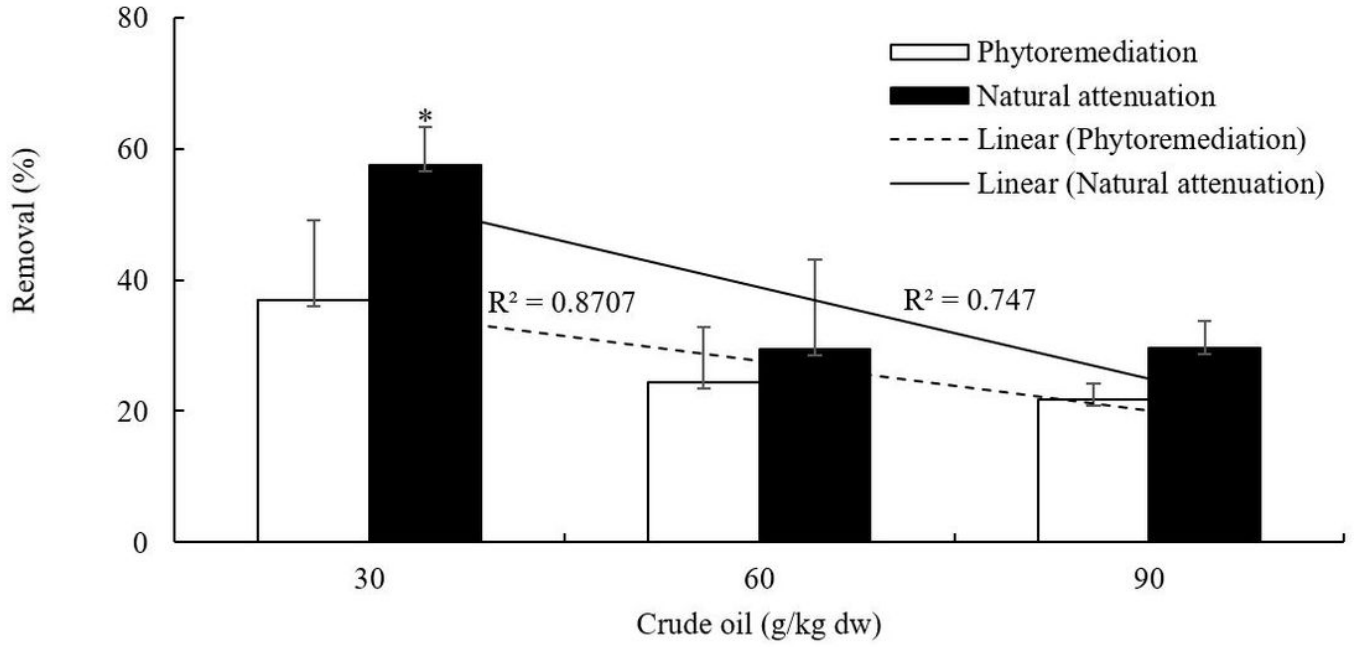

Figure 1

Removal of total petroleum hydrocarbons induced by phytoremediation and natural attenuation (Duncan $p \leq 0.05$, n=6).