Artículo original de Investigación

Phytoremediation of petroleum hydrocarbons-contaminated soil using *Desmodium incanum* DC., Fabaceae

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Abstract

The current research aimed to investigate both the tolerance and the phytoremediation potential of *Desmodium incanum* DC. on petroleum-contaminated soil. There were analyzed *D. incanum seeds* germination, surviving, growth and development cultivated at different contaminants concentrations as well as the pollutant degradation rate by gas chromatography and rhizosphere community. The experiment was carried out on a greenhouse containing non-contaminated soil (NCS), vegetated contaminated soil (VCS) and non-vegetated contaminated soil (NVCS) at the following petroleum concentrations (petroleum per of dry soil): 50 g kg^{-1} and 100 g kg^{-1}. The experiments were performed during 90 days. The germination was more evident as it was observed higher petroleum concentrations. After 90 days, the surviving rate of the both groups 100%, and the soil samples were extracted and analyzed by gas chromatography. The VCS (100 g kg^{-1}) group growth was reduced when compared to the NVCS (100 g kg^{-1}). The petroleum influenced the morph anatomy and development of the plant. Significant increases in the total area, cortical and central cylinder of the roots in the contamination presence. The plant provided the development of larger amounts of microorganisms in the rhizosphere zone, and higher petroleum compounds degradation, confirming their potential phytoremediation for soils contaminated by petroleum.

Key words: *Environmental pollution, Rhizodegradation, Rhizosphere, Legumes.*

Resumen

La investigación tuvo como objetivo evaluar la tolerancia y el potencial de la fitorremediación de *Desmodium incanum* DC., en un suelo contaminado de petróleo. Se analizaron la germinación de las semillas de *D. incanum*, supervivencia, crecimiento y desarrollo de cultivo a diferentes concentraciones del petróleo, así como la tasa de degradación de contaminantes por cromatografía de gases y la comunidad rizosfera. El experimento se llevó a casa del invernadero que contiene los tratamientos con suelo no contaminado (NCS), con vegetación de suelos contaminados (VCS) y sin vegetación en suelos contaminados (NVCS) en las siguientes concentraciones de petróleo (petróleo por suelo seco): 50 g kg^{-1} y 100 g kg^{-1}. Los experimentos se realizaron durante 90 días.

La germinación fue más evidente en las concentraciones de petróleo más altos. Después de 90 días, la tasa de sobrevivencia de los grupos de ambas fueron del 100%, y después las muestras de suelo se extrajeron y analizaron por cromatografía de gases. El VCS (100 g kg^{-1}) presentó crecimiento reduzo en comparación con el NVCS (100 g kg^{-1}). El petróleo influyó en la morfo-anatomía y desarrollo de la planta. Se observó aumentos significativos en la superficie total, cilindro cortical y central de las raíces en la presencia de contaminación. El desarrollo de las plantas en los suelos contaminados por el petróleo, las grandes cantidades de microorganismos en la zona de la rizosfera, y una mayor degradación de compuestos de petróleo, lo confirma su potencial para la fitorremediación de suelos contaminados por el petróleo.

Palabras clave: *Contaminación ambiental, Rizodegradación, Rizosfera, Leguminosas*

1. Introduction

The petroleum is the world's leading energy matrix, however, despite the great importance in the economic panorama presents significant environmental problems (Speight, 2012; 2014; Wang *et al*., 2011), either by contamination of the environment, in industrial and derivatives use, making it one of the largest pollutants the many different ecosystems (Alrumman *et al*., 2015; Bramley-Alves *et al*., 2014; Lotfinasabasl *et al*., 2013; Zhu *et al*., 2015).

When in contact with the environment, the petroleum can be changed in their original characteristics due to physical factors such as the degree of impact of the spill and characteristics of the affected environment, in addition to biological factors (Maranho *et al*., 2006; Speight, 2014). The polycyclic aromatic petroleum hydrocarbons are dangerous due to effects carcinogenic, mutagenic and teratogenic in the organisms, include damages for the health humans (Lau *et al.,* 2014; Sauret *et al.,* 2015; Sun *et al.,* 2010; Wu *et al*., 2013; Zhu *et al*., 2015). Environmental pollution caused by petroleum, either in oceans, lakes, rivers and soils occurs through extraction processes, refining, processing, transportation, abandonment of refinery sites and pipeline ruptures (Malik *et al*., 2012; Peng *et al*., 2009; Soleimani *et al*.,

2010). This leads to ecological damage, causing the death of many animals and plants (Lotfinasabasl *et al*., 2013; Mendez-Natera *et al.*, 2007). When soil contamination occurs, there is a decrease in quality, affecting the availability of water, oxygen and nutrients (Dindar *et al*., 2013; Malik *et al*., 2012).

As for the effects of the affected plants changes occur in weight, height, diameter, leaf area, number of stomata and the potential for photosynthesis (Maranho *et al*., 2006; Nie *et al.*, 2011; Peng *et al.,* 2009). Direct contact with the oil causes the disintegration of the cell membrane and death, reduction of gas exchange and inhibition of germination (Lorestani *et* al., 2014; Sangabriel *et al.*, 2006).

Various techniques have been employed for the treatment of soils contaminated by petroleum, be they physical or chemical (Farias *et al.,* 2009; Peng *et al*., 2009). However, processes are costly and in many cases, uneconomical (Cunninhgam *et al*., 1995; 1996; Soleimani *et al*., 2010; Sun *et al*., 2010). A promising technology from the decade of 1990, the phytoremediation has been emerging and an excellent strategy for the treatment of various contaminants, whether organic or inorganic (Ferrera-Cerrato *et al*., 2007; Gherhardt *et al*., 2009; Peng *et al.,* 2009; Tripathi *et al*., 2015).

The process consists of using plants and microorganisms associated or with the use and production of enzymes, to reduce the contaminants to a level non-toxic to the environment (Ighovie *et al.*, 2014; Tripathi *et al.*, 2015; Wang *et al.*, 2011). This technology has demonstrated high efficiency, besides being considered promising regions of tropical climates, due to the increase of plant growth and microbiological activities (Farias *et al.*, 2009; Merkl *et al.*, 2004).

Among the effective techniques to treat organic contaminants such as petroleum, it is rhizodegradation (Ferrera-Cerrato *et al.*, 2007; Gerhardt *et al.*, 2009). The rhizodegradation refers to the degradation of organic pollutants in the soil by microorganisms developed in the region of rhizosphere (Ali *et al.*, 2013; Maqbool *et al.*, 2012; Nie *et al.*, 2011; Peng *et al.*, 2009; Wang *et al.*, 2011). Rhizosphere provide oxygen, sugars, amino acids, enzymes and other compounds that stimulate the growth and development of microorganisms related, increasing the degradation of contaminants (Ali *et al.*, 2013; Bona *et al.*, 2015; Merkl *et al.*, 2004; Soleimani *et al.*, 2010).

Several plant groups appear as promising in phytoremediation, standing among them, the group known popularly as legumes. These plants have little branched system and little deep roots, allowing operations in deeper layers of the soil (Ferrera-Cerrato *et al.*, 2007). Their symbiosis with nitrogen-fixing bacteria allows the stabilization of soil nutrients, providing benefits to microorganisms associated with plant (Farias *et al.*, 2009)

Inside the group of legumes, the genus *Desmodium* has 350 species that are distributed in tropical and subtropical regions around the world (Ma *et al.*, 2011). Among the species, *Desmodium incanum* DC. ,Fabaceae, has great forage capacity (Hooper *et al.*, 2015) and high

rate of survival in low fertility soils (Granada *et al.*, 2014), being characteristics of great interest when applied in phytoremediation. Thus, the aimed of this study was to evaluate the potential of *D. incanum* for phytoremediation of contaminated soil with petroleum analyzing the responses related to the growth and development of the species, as well as the effects of exposure to contaminants and carry out the determination of hydrocarbon degradation rates in plant treatments, treatments compared to without plants and to study the rhizosphere as well as the effect of petroleum on the population of microorganisms.

2. Material and methods

2.1 Establishment of experiments

The substrate used was collected in Araucaria Forest fragments (Forest with Araucaria), in Curitiba, Paraná, Brazil. Homogenization of the ground was conducted by sieving using sieves of 50 cm of the diameter and 25 mm mesh. There, the established treatments were: non-contaminated soil (NCS); vegetated contaminated (VCS) and non-vegetated (NVCS) contaminated soils in the concentrations (grams petroleum per kilogram of dry soil) 50 g kg^{-1} e 100 g kg^{-1} of petroleum (Farias *et al.*, 2009; Ferrera-Cerrato *et al.*, 2007). The experiments were carried out in a greenhouse, in Universidade Positivo, Curitiba, Paraná, Brazil which showed average temperature of 30 °C. Were used 72 seeds of *Desmodium incanum* DC., collected in a subtropical forest fragment in southern Brazil. Mechanical scarification was made to break of dormancy seeds (Sangabriel *et al.*, 2006) and after seeding three seeds in each pot (Figure 1). This experiment was controlled during 90 days and realized daily irrigations.
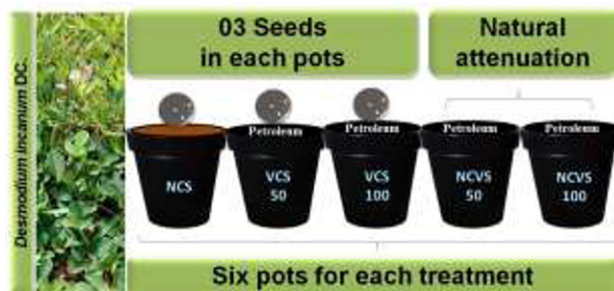
Figure 1. Treatments for the phytoremediation of petroleum contaminated soils using *Desmodium incanum* DC.: non-contaminated soil (NCS); vegetated contaminated (VCS) and non-vegetated (NVCS) contaminated soils in the concentrations (grams petroleum per kilogram of dry soil) 50 g kg⁻¹ and 100 g kg⁻¹ of petroleum

2.2 Evaluation of plant development

During of the 90 days, was analyzed the germination rate, development, presence of chlorosis and survival (Ferrera-Cerrato *et al.*, 2007). For the analysis of biomass, the aerial parts and the roots were separated. After, these materials were placed in stove at 100 °C for 48 hours and after weighted in an analytical balance. Were obtained the biomass before and after for the analyses of reduction water in the material.

2.3 Evaluation of petroleum-effects on morph anatomy roots

The morph anatomy analyses the roots were carried out permanent slides on sections located at the 2 cm from the apex. The samples were fixed with FAA 70 (formaldehyde, acetic acid and ethanol 70%) for 48 hours (Johansen, 1940) and maintained in ethanol 70% until the final processing (Berlyn; Miksche, 1976). For making permanent slides, samples of roots were included in metacrilatoaglicol (JB-4) according to manufacturer's specifications. The sections were done in a rotation micro-tome (Leica RM2125). They were 7 μm thick and were stained with 1% toluidine blue. The obtained illustrations and the scales were made in photomicroscope (Olympus-BX41) by software Image Pro-Plus.

2.4 Study of rhizosphere microorganisms

For the study of the total population of bacteria and fungi were collected soil without plants (NVCS) and soil adhered to the rhizosphere in treatments with plants (VCS). The procedures were performed by the serial dilution method and counting of Colony Forming Units (CFU), presents in petri plates. For dilutions from 10^{-3} to 10^{-5}, were used Dextrose-Papa Agar (PDA) and for dilutions from 10^{-6} to 10^{-8} were uses Nutrient Agar (NA). The petri plates with PDA, were placed in stove at 28 °C for the 48 hours and with NA, in stove at 36 °C for the 48 hours (Ingraham; Ingraham, 1998).

2.5 Efficiency of petroleum degradation on soil

The evaluation of petroleum hydrocarbons degradation was realized in samples collected from rhizosphere soil, though extraction and quantification of the total petroleum hydrocarbons only the highest concentration of treatment (VCS 100 and NVCS 100). The petroleum hydrocarbons were extracted using dichloromethane as solvent under agitation. In the procedure, 2 g of sodium sulfate (Na_2SO_4), 2 g of soil collected and 5 mL of dichloromethane (CH_2CL_2) were added. Subsequently the solution was placed in a shaker at 3000 rpm for 15 minutes and then in a centrifuge (Q-222-T18 / QUIMIS) at 2500 rpm (Schwab *et al.*, 1999).

2.6 Analysis by Gas Cromatography

The extracts were analyzed for gas chromatograph (GC-2010 - SHIMADZU), used a capillary column DB-5 with a diameter of 0.25 micrometres 30 m long and 0.25 mm in width. Hydrogen served as carrier. The initial temperature was 70 °C for 4 minutes, increased to 190 °C (20 °C min^{-1}) to 250 °C. (10 °C min^{-1}) and finally reaching 280 °C (30 °C min^{-1}). For each sample injection, 0.5 uL were applied, and the chromatograms compared based on retention time and area reduction.

2.7 Statistic analysis

Data obtained were tabulated in Microsoft Excel 2013 program spreadsheets, and calculated the means and standard deviations. For the analyzed parameters of the growth plants, morph anatomy of roots, were utilized Test T Student and the efficiency of degradation petroleum hydrocarbons was utilized Test Mann-Whitney by software PAST 2.17c. The significance level considered was $p \leq 0.05$.

3. Results

3.1 Plant development

The germination occurred on the eighth day after seeding in the treatments VCS 50 and VCS 100, which showed higher germination rates, when compared to NCS, with values of 66.7 and 37.5%, respectively (Table 1).

It was found 100% survival rate for the three treatments (Table 1) demonstrating tolerance of the plant front of the petroleum. All treatments presented chlorosis, but VCS 50 and VCS 100 presented higher intensities, with respectively, 75 and 55.6% of frequency. Referring the influence on the growth of *D. incanum*, as increased petroleum concentrations was observed reduction in length and biomass of roots system and aerial parts, and the lengths of internodes (Table 2). For these parameters, it was observed a significant difference compared all treatments ($p < 0.05$).

The reduction in these parameters demonstrated which the petroleum decreases the retention of water and soil nutrients interfering the plant growth and the absorption of nutrients. In contaminated soil, there is formation of a petroleum film around the roots, waterproofing them and impeding the absorption of nutrients.

Table 1. Means ± standard deviations of the germination rate, survival rate, chlorosis frequency of *Desmodium incanum* DC., in response to different concentrations of petroleum.

Parameters	NCS	VCS 50	VCS 100
Germination rate (%)	29.17	66.67	37.50
Survival rate (%)	100.00	100.00	100.00
Chlorosis frequency (%)	42.86	75.00	55.56

3.2 Analysis of the morph anatomy roots

The structural organization of roots was different when compared all treatments, referring to the central cylinders, cortical and total areas (Table 3). For these parameters, it was observed significant difference compared all treatments ($p < 0.05$), the higher the concentration of petroleum, the larger areas obtained.

Table 2. Means ± standard deviations of biomass of roots, biomass of aerial parts, reduction water of roots, reduction water of aerial parts, length of roots, length of aerial parts, length of internodes of *Desmodium incanum* DC., in response to different concentrations of petroleum.

Parameters	NCS (a)	TCP 50(b)	TCP 100(c)
Biomass roots (g)	$0.011 \pm 0.002^{b.c}$	$0.009 \pm 0.002^{a.c}$	$0.006 \pm 0.002^{a.b}$
Biomass aerial parts (g)	$0.029 \pm 0.010^{b.c}$	$0.013 \pm 0.006^{a.c}$	$0.010 \pm 0.004^{a.b}$
Reduction water of roots (%)	45.110 ± 7.720^{b}	31.570 ± 6.310^{a}	57.650 ± 20.750
Reduction water of aerial parts (%)	66.670 ± 4.730^{b}	41.410 ± 15.160^{a}	60.900 ± 12.750
Lenght of roots (cm)	$13.800 \pm 2.413^{b.c}$	$5.480 \pm 1.038^{a.c}$	$2.40 \text{-} 0 \pm 0.943^{a.b}$
Lenght of aerial parts (cm)	$1.770 \pm 0.482^{b.c}$	$1.220 \pm 0.186^{a.c}$	$0.630 \pm 0.111^{a.b}$
Lengths of internodes (cm)	$0.370 \pm 0.114^{b.c}$	$0.220 \pm 0021^{a.c}$	$0.130 \pm 0.020^{a.b}$

Note: Different letters accompanying the figures represent significant difference ($p \leq 0.05$) by Test T Student.

Table 3. Means ± standard deviations of the areas of cylinder central, cortical and total roots of *Desmodium incanum* DC., in response to different petroleum concentrations.

Area of the roots	NCS (a)	VCS 50 (b)	VCS 100 (c)
Cylinder central (µm)	$6980.4 \pm 7393.5^{b.c}$	$17704.0 \pm 6797.1^{a.c}$	$23010.9 \pm 7276.9^{a.b}$
Cortical (µm)	$54873.7 \pm 5819.2^{b.c}$	$271360.3 \pm 143384.5^{a.c}$	$337203.5 \pm 120669.1^{a.b}$
Total (µm)	$61854.1 \pm 7338.2^{b.c}$	$289064.4 \pm 149576.4^{a.c}$	$360214.4 \pm 126691.4^{a.b}$

Note: Different letters accompanying the figures represent significant difference ($p \leq 0.05$) by Test T student.

The root of *D. incanum* (Figure 2A), has uniseriate epidermis and 4-5 layers of aerenchyma, composed of cylindrical/rectangular cells, which may or may not have irregular shapes. The cylinder central (Figure 2D) is the triarch type, and the xylem, surrounded by sclerenchyma fibers.

As the petroleum concentration increased, morphological changes occurred mainly related to changes in intracellular spacing and organization of vascular system. In the VCS 50, there was an increase in the size of the area (Figure 2B), epidermal thickening, increased to 5-8 layers of aerenchyma (Figure 2G) and disorganization of the xylem distribution (Figure 2E), but, kept triarch structure. In relation to the VCS 100, there was an increase in the surface area (Figure 2C) when compared to treatment NCS and VCS 50. A greater thickening of the epidermis, increased to 8-11 layers aerenchyma (Figure 2H), increased intracellular space, and disorganization the xylem distribution (Figure 2F), losing triarch structure presented by the vascular system.
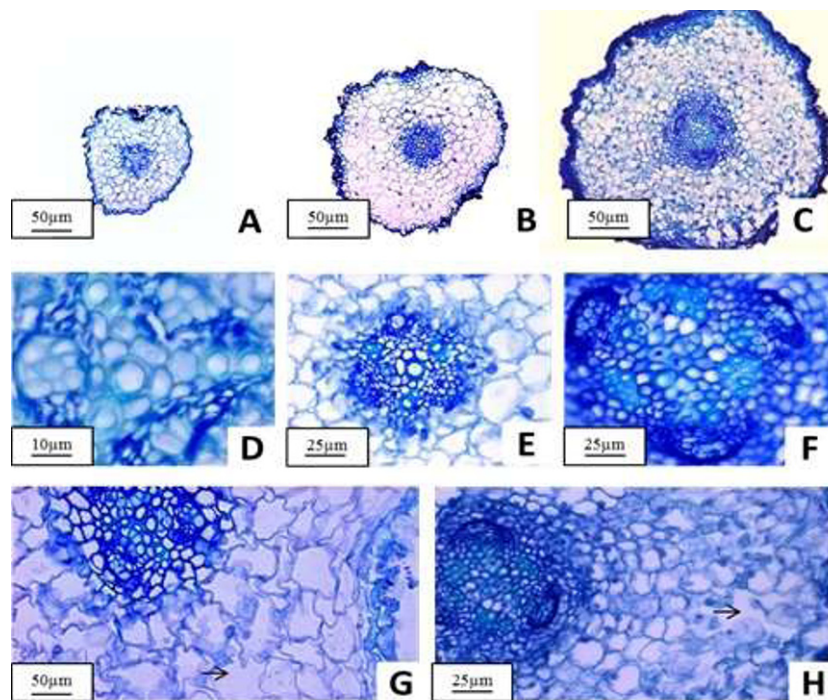
Figure 2. Roots structural Organization of *Desmodium incanum* DC. in response to differents petroleum concentrations **(A)** NCS; **(B)** VCS 50; **(C)** VCS 100; **(D)** Xylem vascular cylinder thriarc of roots NCS; **(E)** Xylem structural disorganization in the cylinders vascular thriarc of treatment VCS 50; **(F)** Xylem total disorganization of treatment VCS 100; **(G)** Aerenchyma of treatment VCS 50; **(H)** Aerenchyma of treatment VCS 100. (→) Indicates of aerenchyma.

3.3 Population of the rhizosphere's microorganisms

The analysis of microorganisms from the rhizosphere of *D. incanum* was performed with all treatments contaminated with petroleum, both with and without plant plant, confirming the growth of fungi and bacteria for all. Was observed a higher quantity fungi in NA medium than in PDA medium (Table 4), but, no significant differences was observed.

In the plates containing PDA medium occurred intense proliferation of bacteria during the period kept in the stove, making the uncountable plates. Therefore, only the fungus population has been accounted for in the 10^{-3} to 10^{-5} dilutions. Referring to bacteria, an increase in the number of CFUs in the treatments with plant (Table 5).

3.4 Degradation of petroleum hydrocarbons

In relation to the petroleum hydrocarbons degradation of (Figure 3A), a reduction the areas of the compounds both treatments has been observed, with plant (VCS100) (Figure 3B), and without the plant (NVCS100) (Figure 3C).

However, when the area reduction observed between treatments, it was noted that the presence of *D. incanum* (VCS 100) was more efficient than the treatment without the plant (NVCS 100), where it was found degradation rate of 9.9 to 66.9% in the compounds analyzed. Table 6 demonstrate that there is a high occurrence of degradation of petroleum hydrocarbons, with difference significative for the 80% compounds analyzed ($p \leq 0.05$).

Table 4. Means ± standard deviations of quantification of fungi presents in the rhizosphere of the *Desmodium incanum* DC., in response to different concentrations of petroleum.

Dilutions	NVCS 50	VCS 50	NCVS 100	VCS 100
10^{-3} (CFU mL^{-1})	0 ± 0	$4 \times 10^2 \pm 4 \times 10^2$	$1 \times 10^2 \pm 1 \times 10^2$	$1 \times 10^2 \pm 1 \times 10^2$
10^{-4} (CFU mL^{-1})	$1 \times 10^2 \pm 5 \times 10^1$	0 ± 0	0 ± 0	0 ± 0
10^{-5} (CFU mL^{-1})	0 ± 0	0 ± 0	$1 \times 10^2 \pm 1 \times 10^2$	$1 \times 10^2 \pm 1 \times 10^2$
10^{-6} (CFU mL^{-1})	$7 \times 10^2 \pm 7 \times 10^2$	$3 \times 10^2 \pm 8 \times 10^1$	$4 \times 10^2 \pm 1.5 \times 10^2$	$3 \times 10^2 \pm 5 \times 10^1$
10^{-7} (CFU mL^{-1})	$1 \times 10^2 \pm 1 \times 10^2$	$2 \times 10^2 \pm 8 \times 10^1$	$1 \times 10^2 \pm 1 \times 10^2$	$2 \times 10^2 \pm 1.5 \times 10^2$
10^{-8} (CFU mL^{-1})	0 ± 0	$1 \times 10^2 \pm 5 \times 10^1$	$1 \times 10^2 \pm 1 \times 10^2$	$1 \times 10^2 \pm 1 \times 10^2$

Table 5. Means ± standard deviations of bacteria's CFUs presents in the rizosphere of the *Desmodium incanum* DC., in response to different petroleum concentrations.

Dilutions	NCVS 50	VCS 50	NCVS 100	VCS 100
10^{-6} (CFU mL^{-1})	$1.08 \times 10^4 \pm 4.20 \times 10^3$	$1.34 \times 10^4 \pm 8.00 \times 10^3$	$8.50 \times 10^3 \pm 1.20 \times 10^3$	$3.15 \times 10^4 \pm 5.80 \times 10^3$
10^{-7} (CFU mL^{-1})	$4.90 \times 10^3 \pm 5.00 \times 10^2$	$1.83 \times 10^4 \pm 1.44 \times 10^4$	$3.20 \times 10^3 \pm 1.10 \times 10^3$	$8.80 \times 10^3 \pm 2.00 \times 10^2$
10^{-8} (CFU mL^{-1})	$5.00 \times 10^2 \pm 2.00 \times 10^2$	$3.50 \times 10^3 \pm 2.70 \times 10^3$	$3.00 \times 10^2 \pm 1.00 \times 10^2$	$8.00 \times 10^2 \pm 4.00 \times 10^2$

Table 6. Means ± standard deviation of the areas values of petroleum compounds, in the treatments NVCS 100 and VCS 100, degradation efficiency and differences statistics.

Peak	Retention Time (min)	Petroleum Area	NCVS 100 Area	NCVS 100 % of reduction	VCS 100 Area	VCS 100 % of reduction	Degradation efficiency (%)	Value of p
1	2.016	1856266.9	14858.8 ± 6581.4	99.20	7637.4 ± 3227.8	99.59	48.6	0.17
2	3.021	408377.6	5717.9±3308.3	98.60	1893.5 ± 771.6	99.54	66.9	0.04*
3	3.234	458849.8	8381.4 ± 4718.2	98.17	3133.2 ± 1911.8	99.32	62.6	0.04*
4	3.694	374771.1	8834.0 ± 4762.2	97.64	3118.2 ± 1433.8	99.17	64.7	0.03*
5	3.804	303156.9	4193.5 ± 2122.4	98.62	1905.6 ± 964.3	99.37	54.6	0.05*
6	4.051	174861.2	4213.9 ± 2092.6	97.59	1635.0 ± 347.1	99.06	61.2	0.01*

7	4.985	900277.1	23508.6 ± 12366.0	97.39	7989.5 ± 2644.7	99.11	66.0	0.01*
8	5.528	645658.8	17459.6 ± 9452.9	97.30	5856.9 ± 1989.6	99.09	66.4	0.01*
19	6.357	285603.9	8871.6 ± 3742.5	96.89	3868.1 ± 1037.0	98.65	56.4	0.01*
10	6.795	913698.9	31225.5 ± 12456.2	96.58	12326.6 ± 3882.1	98.65	60.5	0.01*
11	6.905	454470.5	15251.5 ± 5916.1	96.64	7228.2 ± 2052.4	98.41	52.6	0.01*
12	6.976	381446.3	13357.8 ± 5072.6	96.50	7261.8 ± 2069.4	98.10	45.6	0.03*
13	7.211	361775.8	12331.9 ± 4526.7	96.59	6845.3 ± 2225.6	98.11	44.5	0.03*
14	7.298	1857743.4	78936.4 ± 28338.9	95.77	40328.7 ± 9771.2	97.83	48.9	0.01*
15	7.436	761597.8	28316.1 ± 9708.9	96.28	18080.6 ± 4881.3	97.63	36.1	0.03*
16	11.816	624469.7	16626.2 ± 16228.9	97.33	6399.2 ± 2525.3	98.99	61.5	0.05*
17	12.596	220627.1	6397.4 ± 3015.6	97.10	3886.0 ± 903.9	98.24	39.3	0.26
18	13.319	320902.6	9485.5 ± 3644.0	97.04	8009.7 ± 4901.3	97.50	15.6	0.04*
19	14.621	334821.8	11409.4 ± 3203.8	96.59	10271.6 ± 8610.1	96.93	9.9	0.57
20	15.656	314877.5	11270.5 ± 6565.9	96.42	5123.3 ± 3765.9	98.37	54.5	0.10

Note: $^{(*)}$ Accompanying represent significant difference ($p \leq 0.05$) by Mann-Whitney Test.
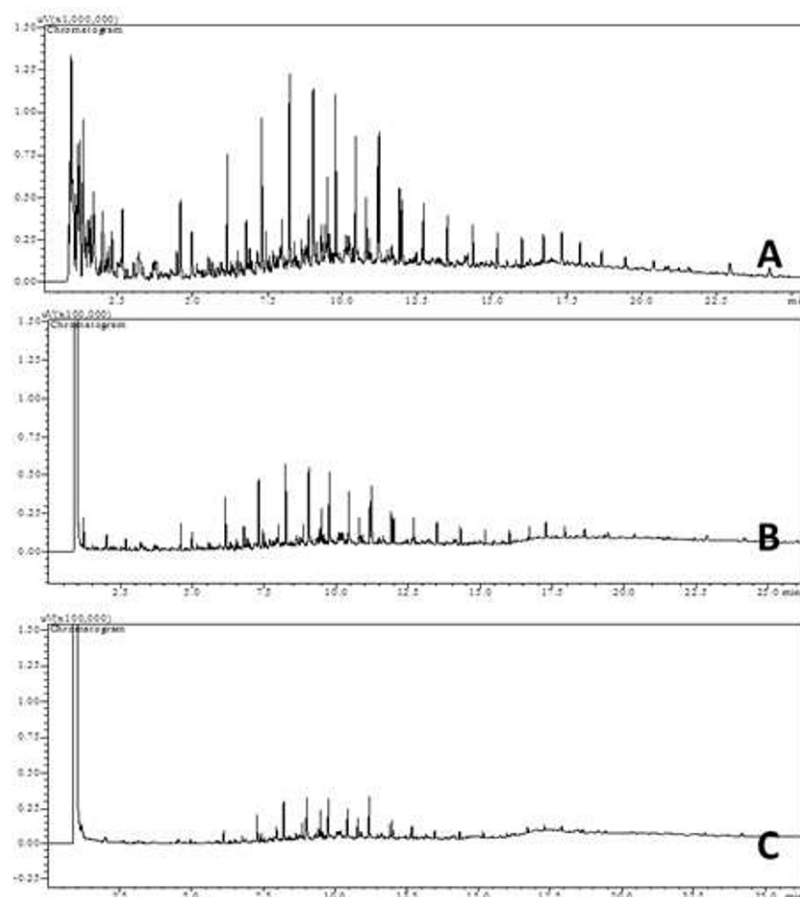
Figure 3. Chromatograms obtained by gas chromatography, demonstrating the peaks of petroleum compounds**. A)** Chromatogram of pure petroleum; **B)** Chromatogram demonstrating the degradation of petroleum compounds using *Desmodium incanum* DC. (VCS 100); **C)** Chromatogram demonstrating the degradation of petroleum compounds without plant (NVCS 100) to evaluate the natural attenuation.

4. Discussion

The contamination by petroleum hydrocarbons affects on the germination of seed plants due to physical and chemical changes that promote effects on their development. In this study, it was demonstrated that the seeds germination of *D. incanum* presented responses positives in the presence of petroleum, as also observed by Farias *et al.* (2009), which *Erythrina crista-galli* L., Fabaceae. These authors demonstrated higher germination rates of *E. crista-gall* seeds when in contact with the petroleum. Others authors demonstrate that petroleum can act positively on seed germination, acting as auxin, helping the germination process (Rivera-Cruz; Trujillo-Narcía, 2004).

This facts demonstrated the tolerance of the *D. incanum* to petroleum contaminated soil. In the works of the Sangabriel *et al.* (2006) and Ferrera-Cerrato *et al.* (2007), it was also observed the tolerance and survival of the leguminous specie, *Phaseolus coccineus*, when exposed to petroleum-contaminated soil.

Were observed reduction of the parameters of biomass, length and reduction of the water of the aerial parts and roots. This was also observed in the work of Ferrera-Cerrato *et al.* (2007), Merkl *et al.* (2004) and Nie *et al.* (2011). According to Maranho *et al.* (2006), the petroleum decreases the retention of water and soil

nutrients interfering in the plant growth. This can be demonstrated by a significant reduction in water aerial parts (p < 0.005) and root (p < 0.02) compared to treatment VCS 50 with NCS. These differences are related to interference caused by the petroleum in the water distribution process in the structure plant (Merkl *et al.*, 2004), promoting changes such as increasing the diameter of the root and reducing its growth (Farias *et al.*, 2009).

The roots presented larger areas in the higher the petroleum concentration. It was observed further development of intercellular spaces (aerenchyma) when the roots were exposed to higher concentrations of petroleum. This allows that oxygen and other gases diffuse through the cells, allowing tolerance and survival of plants in contact with the pollutant. Furthermore, it was observed disorganization of the central cylinder of the roots when exposed to higher concentrations of petroleum. Changes in the central cylinder in response to different petroleum concentrations were also observed by Farias *et al.* (2009) to *E. crista-galli*.

The morphological alterations in the roots can affect the degradation promoted by microorganisms (Farias *et al.*, 2009), impairing or contributing the effects of phytoremediation in the contaminant (Merkl *et al.*, 2005). In response to depletion of oxygen in the soil, due the petroleum contaminated, there is the development of aerenchyma, contributing to gas diffusion to the roots (Yamauchi *et al.,* 2013). This fact contributing for the degradation of pollutants because there favoring the development of degrading microorganisms.

The petroleum alters soil conditions, waterproofing it by promoting deficiency of water and interference in the uptake by the plant (Merkl *et al.*, 2005). Facing this water stress, there is a morphological adaptation of increased root diameter and reduced length, in order to enlarge the contact surface and water absorption (Farias *et al.*,

2009). Farias *et al.* (2009) and Merkl et al. (2005) describe that morphological and anatomical changes may promote root growth and development of microorganisms that degrade petroleum.

In the treatments with plants, it was observed a greater diversity of color and morph types of microorganisms and was observed further development of fungi and bacteria. This fact was also observed in the work of Ferrera-Cerrato *et al.* (2007), which recorded the highest quantity of rhizosphere microorganisms of *Phaseolus coccineus*. Moubasher *et al.* (2015), cited which where plants, were present the highest counts of fungi and bacterias in the roots zone.

Bramley-Alves *et al.* (2014) found that the presence of *Poa foliosa* there is an increased of petroleum bacteria degrading. The observed may be explained due to the root system of the plants permit rapid movement and carrying water and gases into the ground (Soleimani *et al.*, 2010). Furthermore, the release of different substances exuded by the roots of plants contributes to the growth of microorganisms in the rhizosphere (Merkl *et al.*, 2005). In accordance to Moreira (2013), the presence of larger amounts of microorganisms in plant treatments, demonstrating the presence of phytoremediation potential.

It was observed difference significate (p ≤ 0.05) in the degradation with *D. incanum,* principally for petroleum hydrocarbons of low weight. It was associated to the highest development of microorganisms in treatments with plants (VCS), which contributed with biorremediation for biodegradation, as observed by Wang *et al.* (2011) when study the rhizodegradation using different plants.

Merkl *et al.* (2005) observed a significant reduction to the aromatic compounds in presence of *Brachiaria briazantha* (Hochst. Ext. A. Rich.), which suggesting correlations with the changes observed in the roots, with the potential degrading. The plants have prompted significant changes

through the rhizosphere favoring the degradation process (Ferrera-Cerrato *et al.*, 2007).

Ferrera-Cerrato *et al.* (2007) and Farias *et al.* (2009) also observed higher degradation of petroleum compounds using leguminous plants, *Phaseolus coccineus* and *Erythrina crista-galli*, respectively. In the evaluation of potential of the *Sesbania cannabina* (Fabaceae) for the petroleum hydrocarbons degradation, Maqbool *et al.* (2012) verified which the high subsurface area of roots helps in the proliferation of rhizospheric microbial community, which contributes to the treatment.

In the present study, it was observed a maximum reduction of petroleum total hydrocarbons (up to 97%), using *D. incanum*. Peng *et al.* (2009); it was also observed a higher degradation in treatments using plants, as in the present study, because *Mirabilis jalapa* presented maximum reduction of petroleum total hydrocarbons up to 63.30%, lower values when compared with the present research.

Efficiency in the treatments with plants (VCS 100) occurred when analized the composts of low weight molecular and high weight. Moubasher *et al.* (2015) observed that the microorganisms' activities contributed for the degradation petroleum hydrocarbons. But, the efficiency of the degradation petroleum hydrocarbons in unplanted treatments occurred generally by volatilization, photoxidation or the activity of its original microorganisms of soil (Moubasher *et al.,* 2015; Peng *et al.,* 2009; Sun *et al.,* 2010).

This study demonstrated the potential of the *Desmodium incanum* for the petroleum degradation by phytoremediation, due the increase of roots in biomass, length, aerenchyma and development of microbial community. Thus, understanding of the interaction between plants and microbes is important to select those that exhibit the greatest potential for phytoremediation of contaminated soil by petroleum.

5. Conclusions

The results obtained in this study demonstrate that *Desmodium incanum* DC. is tolerant to soil contaminated by petroleum, with high rates of survival and better germination performance when in contact with the contaminant. Structural changes could be observed as a survival strategy, in which the plant mainly introduced modifications in its root structure, as the length reduction and diameter increase. We also observed, most petroleum compounds degradation and greater quantity of rhizosphere microorganisms in plant treatment than without treatment plant. These results and observations thus allow the inference that *D. incanum* has phytoremediator potential and can be used in the tretament soil contaminated by petroleum.

6. Acknowledgements

7. References

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