Petroleum Hydrocarbons Rhizodegradation by *Sebastiania commersoniana* (Baill.) L. B. SM. & Downs

Débora Toledo Ramos · Leila Teresinha Maranho · Ana Flávia Locateli Godoi ·
Marco Aurélio da Silva Carvalho Filho · Luiz Gustavo Lacerda ·
Eliane Carvalho de Vasconcelos

Abstract Petroliferous activities in Brazil have an accelerated development in the last years. As a consequence, the incidence of environmental accidents such as oil spills and contamination of soils has increased significantly. Therefore, it is extremely necessary to develop remediation techniques with lower costs, decontamination efficiency and impact minimisation. The aim of this work was to evaluate *Sebastiania commersoniana* phytoremediation potential in soil contaminated by petroleum. This species, which is a native tree, was selected due to its proven capacity for surviving in areas contaminated by petroleum. Experiments were carried out with soils that were vegetated but non-contaminated, soils that were freshly contaminated (25, 50 and 75 g kg^{-1}) but non-vegetated, and soils that were vegetated and contaminated, samples were collected 60 and 424 days after contamination with the purpose of evaluating the percentage of petroleum degradation in relation to the time. The results obtained in the present study allow us to state that *S. commersoniana* proves to be tolerant to petroleum contamination with respect to plant's growth. The degradation of petroleum hydrocarbons was evaluated by gas chromatography with a flame ionisation detector (GC-FID) equipped with a capillary column HP-5 (5% phenyl-methylpolysiloxane, 30 m; 0.25 mm; 0.25 µm). According to chromatographic analysis, samples in contact with *S. commersoniana* showed a significant area reduction of the hydrocarbon peaks. Analysis of the 60-day samples showed a reduction of petroleum hydrocarbons area higher than 60% and the 424-day samples showed a reduction higher than 94%, which demonstrates that a petroleum degradation process is occurring.

Keywords Phytoremediation · Petroleum ·
Rhizodegradation · Pollution · Rhizosphere

D. Toledo Ramos (✉) · L. T. Maranho · A. F. L. Godoi ·
M. A. S. Carvalho Filho · E. C. de Vasconcelos
Post-Graduation in Environmental Management,
Positivo University,
Curitiba, PR, Brazil
e-mail: debora.toledo@uol.com.br

L. G. Lacerda
Post-Graduation in Bioprocess Engineering
and Biotechnology Division, Federal University of Parana,
Curitiba, PR, Brazil

A. F. L. Godoi
Environmental Engineering Department,
Federal University of Paraná,
P.O. Box 19011, 81531-990 Curitiba, PR, Brazil

1 Introduction

Soils contamination by organic pollutants accumulation is a widespread issue. Contaminated soils are found on all continents. Accidental spills occur mainly in areas near oil refineries, storage areas and significant manufacturing activities. Specific techniques, aiming to remediate these areas, consume considerable economic resources of industries and governments (Cunningham et al. 1996).

There are a set of methods to remediate soils such as chemical and physical methods, but the great concern of governments and industrial sector is the cost these techniques may represent to their budget. For this reason, it is extremely necessary to develop new alternative techniques of remediation which might offer not only efficiency and impact minimisation but also lower costs (Dietz and Schnoor 2001).

When petroleum enters in the environment, it starts to changes its original characteristics due to abiotic factors (humidity, pH, temperature, etc.) and biotic factors (degradation). These changes are regulated by specific features of the spill and also by the affected environment (Maranho, 2004). For this reason, petroleum may persist in the environment for a long period of time and due to the slow biodegradation process of hydrocarbons (Michel et al. 2002).

Bioremediation is the main mechanism of petroleum degradation in phytoremediation and it occurs by stimulating microbial growth on the biodegradable fraction of the organic contamination that is susceptible to microbial attack (Hernández-Acosta et al. 1997; Shen and Bartha 1996). Due to the presence of exudates, such microorganisms are widely spread in the soil rhizosphere where the degradation process is presumed to occur (Roitman et al. 1991; Gentry et al. 2004; Phillips et al. 2006; Euliss et al., 2008; Escalante-Espinosa et al. 2005; Lin and Mendelssohn 1998).

Phytoremediation is a relatively new technique in which the plant is directly or indirectly responsible for removing soil contaminants. This technique presents several advantages such as: low cost of operation and implementation, efficiency, environmental friendliness and applicability to a wide range of pollutants. Moreover, as it is an "in situ" technique, it minimises the risk of impacting the area when the contaminated layer is removed.

One of the main drawbacks of phytoremediation is the necessity of identifying species with phytoremediation potential, as only a few of them are able to tolerate and grow in contaminated areas (Merkl et al. 2004).

At the present work, the selected plant species was *Sebastiania commersoniana* (Baill.) L. B. SM. & Downs, also known as "branquilho", is a native tree of medium size from the Euphorbiaceae family. It was chosen because it occurs naturally in the forests of Southern Brazil and it has survived in an area where an accidental spill of four million litres of oil took place. After the accident, it was observed that this species was one of the few plants which managed to endure, proving its capacity for tolerating petroleum.

The influence of petroleum hydrocarbons on plants has been reported by several authors (Davies and Bacon 2003; Merkl et al. 2005; Maranho et al. 2006a; Kechavarzi et al. 2007), demonstrating that the toxic effects of petroleum can affect the plant's leaves and root system, decreasing oxygen availability and, consequently, inhibiting plant growth and development.

Maranho et al. (2006b) showed that some morphological changes occurred with *S. commersoniana* while the plant was exposed to petroleum toxicity such as development of new roots, increase in the number of intercellular spaces and increase of stomates density. These morphological changes are characterised as metabolic strategies that can enhance the amount of oxygen provided to the plant rhizosphere, so that microbial communities are stimulated and start to degrade petroleum hydrocarbons. However, those authors did not evaluate the plant's phytoremediation potential for degrading petroleum hydrocarbons, justifying the present work. The aim of this work was to evaluate *S. commersoniana* phytoremediation potential to degrade petroleum hydrocarbons from soils.

2 Materials and Methods

2.1 Experiment Preparation

The soil used in the experiments was obtained in a contamination-free area next to the co-ordinate axes 25°34′02.5″S and 49°20′53.5″W, at 910 m above sea level. The soil is characterised by presenting a 6.5 pH, as well as density of 26.9 $g.dm^{-3}$ and a clayish texture. Experiments were carried out in a greenhouse at a temperature of 25–30°C and relative humidity of 85–90% with soil that was vegetated and non-contaminated (SVNC), soil that was non-vegetated and contaminated (SNVC) and soil that was vegetated and contaminated (SVC). Treatments were monitored for 424 days, with the purpose of evaluating the percentage of petroleum degradation in relation to the time. The contamination of soil samples with petroleum was done in the university laboratory in three different concentrations: 25, 50 and 75 g of petroleum/kg of soil (25, 50 and 75 $g\ kg^{-1}$, respectively). These concentrations were chosen according to the

concentration used in articles of the same research area (Ferrera-Cerrato et al. 2006; Sangabriel et al. 2006). After applying the petroleum to the soils and homogenising it all, the prepared soils were placed in 22 cm×24 cm wide pots. Seeds of *S. commersoniana* were sowed in each of the pots (one seed per pot). The germination percentage was 87% for the treatment SVC 75 g kg^{-1} whereas for the treatments SVNC, SVC 25 g kg^{-1} and SVC 50 g kg^{-1} the germination rates were 85%, 75% and 80%, respectively. The pots were irrigated twice a day (8 a.m. and 6 p.m.) for 1 h—as this species is used to areas highly irrigated. The water used was treated-water provided by the Water Treatment Station of Curitiba, Paraná, Brazil.

2.2 Soil Samples Collection

Samples of soils were collected after 60 and 424 days. The samples were collected in the rhizosphere region (3–5 mm) and transferred to small sterile plastic pots and stored in a refrigerator at a temperature of 5ºC.

2.3 Plant Analysis

In order to make a complete analysis of the toxic effects of petroleum on the plant, morphological analyses such as plant height and the number of leaves and branchings were conducted. During September and October (eighth and ninth months of experiment), analyses were performed on a weekly basis. During November (tenth month of experiment) two analyses were carried out and from December to February (11th and 13th months of experiment) analyses were performed monthly. Plant height analysis was performed with the aid of a ruler and a measuring tape.

After 424 days, at the end of the experiment, plants were collected from the pots and their roots were separated from the aerial part so that analysis of root volume, humidity content and dry biomass could be performed. Root volume analyses were carried out using the displaced volume method, which consists of introducing a known volume of distilled water into a test tube and after the roots are introduced into this test tube, the displaced volume is recorded. The displaced volume corresponds to the volume of roots (Ferrera-Cerrato et al. 2006). Humidity content and dry biomass analyses were carried out by first weighting the samples and then using drying processes in a greenhouse with forced air circulation, at a temperature

of 72ºC during 24 h until a constant sample weight was established. The dry biomass was the weight at the end and the difference in weights before and after drying was considered to be due to water (humidity) loss.

2.4 Statistics Analysis

Data obtained in the following analysis: plant's medium height, humidity content, dry biomass, roots volume and pairs of leaves were submitted to the variance analysis (ANOVA) double factor, followed by the Fisher LSD test. All tests were performed by the programme Statistica for Windows® version 6.1 and it was considered 5% of data significance ($p \leq 0.05$), aiming to demonstrate how much the variables differ between the experimental groups (SVNC and SVC).

2.5 Extraction Process and Chromatographic Analysis

Petroleum extraction from the soil samples was carried out using dichloromethane as the solvent. In a glass flask, 2 g of soil sample and the slurry were submitted to extraction using three portions of 5 mL of dichloromethane and each 5-mL-portion was agitated for 1 h. Afterwards, samples were united in the same vial and centrifuged at 2,500 rpm for 15 min. Then, the supernatant was collected with a Pasteur pipette and transferred to an amber vial, which was stored in the freezer until it was analysed.

Chromatographic analysis was performed using a Shimadzu GC-FID (gas chromatograph–flame ionisation detector) equipped with a capillary column HP-5 (5% phenyl-methylpolysiloxane, 30 m; 0.25 mm; 0.25 μm), with the following temperature programme: starting at 70ºC and heated until 190ºC at 20ºC min^{-1}, to 250ºC at 10ºC min^{-1}, and finally to 280ºC at 30ºC min^{-1}, then holding for 10 min. Hydrogen was used as carrier gas at 1.0 mL min^{-1}. Injector and detector temperatures were 250ºC and 280ºC, respectively. Additionally, a GC-MS (gas chromatograph–mass spectrometer) analysis was performed and the chromatograph was equipped with a capillary column HP-5 (5% phenyl-methylpolysiloxane, 30 m; 0.25 mm; 0.25 μm), with the following temperature programme: starting at 70ºC then heated until 190ºC at 5ºC min^{-1}, to 250ºC at 5ºC min^{-1}, and finally to 280ºC at 5ºC min^{-1}, then holding for 20 min. Helium was used as the carrier gas at 0.9 mL min^{-1}. Injector and detector temperatures were 250ºC and 280ºC, respectively.
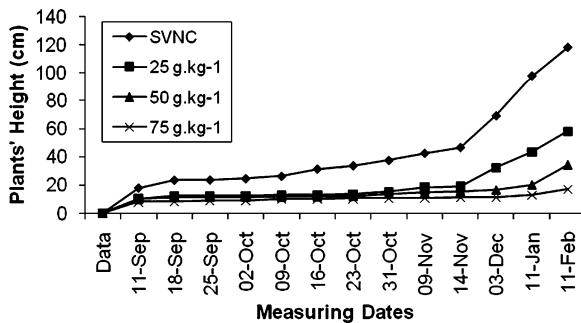
Fig. 1 Dependence of plants' medium height in contact with different petroleum concentrations

Samples were injected in triplicate and the volume injected was 0.5 μL. Through the chromatograms, peaks representing the homologous series of petroleum were selected. Chromatograms from freshly contaminated (zero time), 60- and 424-day samples were compared, taking into account the homologous series peak area, aiming to evaluate whether a reduction of the peak areas occurred or not.

2.6 Rhizosphere Microbial Analysis

Microbiological analysis was performed with the purpose of evaluating microbial density and diversity in the soil samples. The analyses were performed with samples of groups SVNC and SVC, using two culture media: plate count agar for enumerating bacteria and dichloran rose bengal chloramphenicol agar (DRBC) for enumerating fungi (Sangabriel et al. 2006; Silva et al. 2001). Two grammes of soil samples were added to a sterile flask containing a sterile solution of sodium chloride 0.9% that was used as diluent and then serial dilutions were conducted.

Plate inoculation was done using the spread plate technique, and the incubation temperature was 28ºC (82.4ºF) for fungi and 36ºC (96.8ºF) for bacteria. Fungi colony formation units (CFU) were enumerated after 7 days and bacteria CFU were enumerated after 3 days (Silva et al. 2001; Sangabriel et al. 2006).

3 Results and Discussion

3.1 Plant Analysis

S. commersoniana revealed to be tolerant to the stress imposed by petroleum presence. However, plant growth was inversely proportional to an increase in petroleum concentration as a result of the toxicity caused by the pollutant to the plant.

Plants that were placed in pots containing soil with higher concentrations of petroleum had poorer development in comparison with those in contact with lower concentrations of the pollutant (Fig. 1).

Ferrera-Cerrato et al. 2006 and Sangabriel et al. 2006 observed a reduction in the growth of the majority of species that were kept in contact with petroleum-contaminated soil when compared to those that were kept in a non-contaminated soil.

S. commersoniana's growth was slow during September and October (spring time), showing a slight growth increase in November and growth acceleration from December on (summer time; Fig. 1). Such results, according to Lorenzi (2002); Solon et al. (2005), are related to the physiological features of the studied species, as it intensifies its growth mainly from September to December. An inhibition of the growth and development of the plants by the presence of petroleum hydrocarbons was noticed when compared to the behaviour of individuals that grew up in non-contaminated soils. Such inhibition in growth may be caused by the absorption of petroleum small toxic molecules which may alter the integrity and permeability of plant membranes (Reis 1996).

At the beginning of the experiment, it was possible to count the number of pairs of leaves (Fig. 2), though as the plant was developing, the number of pairs of leaves increased significantly, until it was impractical
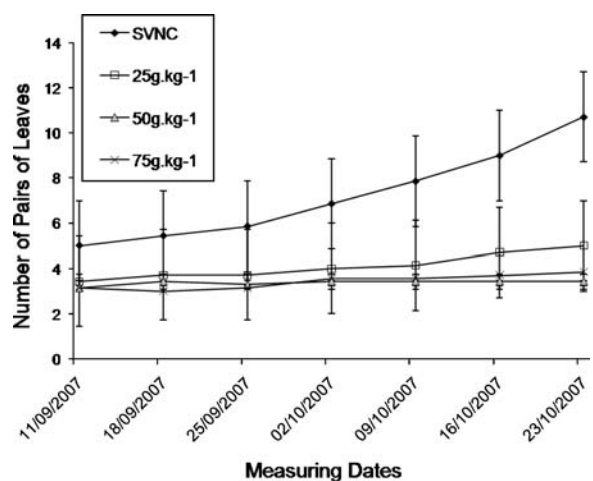

Fig. 2 Dependence of plants' number of pairs of leaves in contact with different concentrations of petroleum
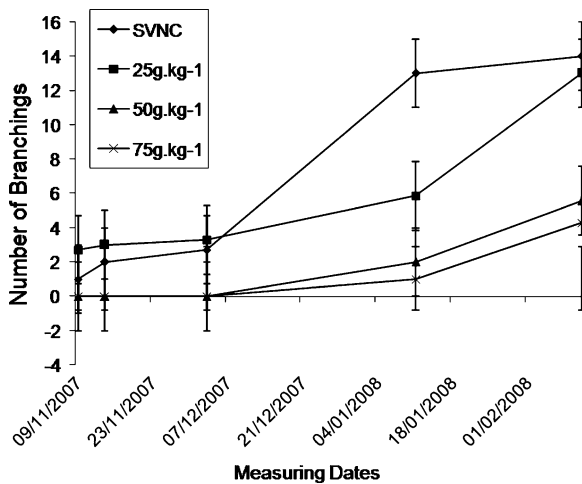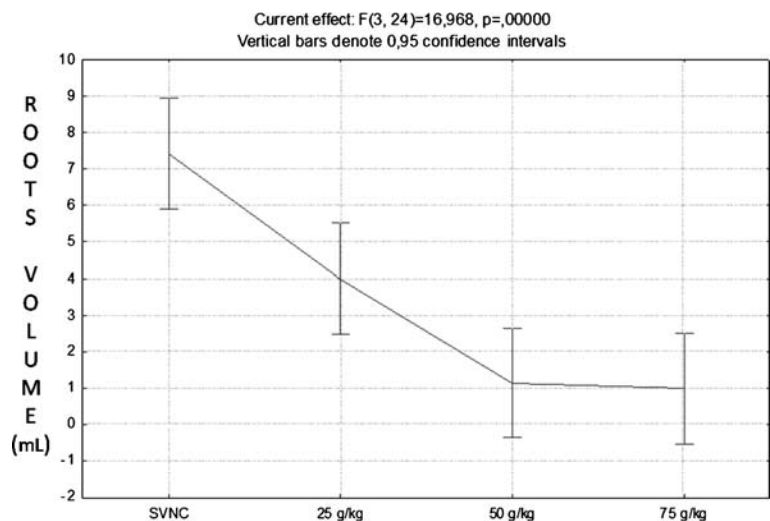
Fig. 3 Dependence of plants' number of branchings in contact with different concentrations of petroleum

to count them, so later the plant branching were counted instead (Fig. 3).

Both analyses were performed with the same frequency as the plant height analyses. An inversely proportional relation was observed between the number of pairs of leaves and branchings and petroleum concentration, owing to the stress caused by petroleum during the development of the plant. An increase in the number of pairs of leaves and branchings in relation to time that was proportional to plant growth was also noted. Such results can favour the decontamination process because the increase in the number of leaves might represent a higher number of stomata present in plant leaves

available to make oxygen capitation and diffusion to the rhizosphere and, as a consequence, stimulating aerobic microbial growth, enhancing rhizodegradation (Maranho et al. 2006a).

Plant roots were analysed at the end of the experiment due to the necessity of taking the plants out of the pots to collect them. It was noticed that plants kept in contact with higher petroleum concentrations had a poor development of their roots when compared to the plants that grew up in non-contaminated soil (Fig. 4).

This poor development of plant roots may be attributed to petroleum hydrophobicity which prevents water from being uniformly distributed in the contaminated soil, resulting in a hydric deficiency (Merkl et al. 2004). Aiming to overcome such deficiency, the plant develops an adaptation mechanism and starts to increase the diameter of the roots and reduce its length (Kechavarzi et al. 2007).

The negative influence of petroleum hydrocarbons towards plants' roots development was reported by several studies (Palmroth et al. 2002; Hou et al. 2001). Additionally, changes in root morphology can reduce water and nutrient capitation by plants and, consequently, can inhibit their growth and development (Merkl et al. 2005), justifying the above results.

It was observed that individuals in contact with the highest petroleum concentration contained lower humidity content in their composition in comparison with those in contact with lower concentration or in the absence of petroleum (Fig. 5). This may be attributed to the hydrophobic nature of petroleum

Fig. 4 Dependence of plants' roots volume in contact with different concentrations of petroleum. Comparison between averages obtained for the roots volume analysis through variance analysis (ANOVA). *Vertical bars* indicate 0.95 of confidence intervals

Current effect: $F_{(3, 24)}=16,968$, p=,00000
Vertical bars denote 0,95 confidence intervals

Fig. 5 Relation between humidity of plants' aerial part in contact with different petroleum concentrations. Comparison between averages obtained for the humidity content analysis through variance analysis (ANOVA). *Vertical bars* indicate 0.95 of confidence intervals
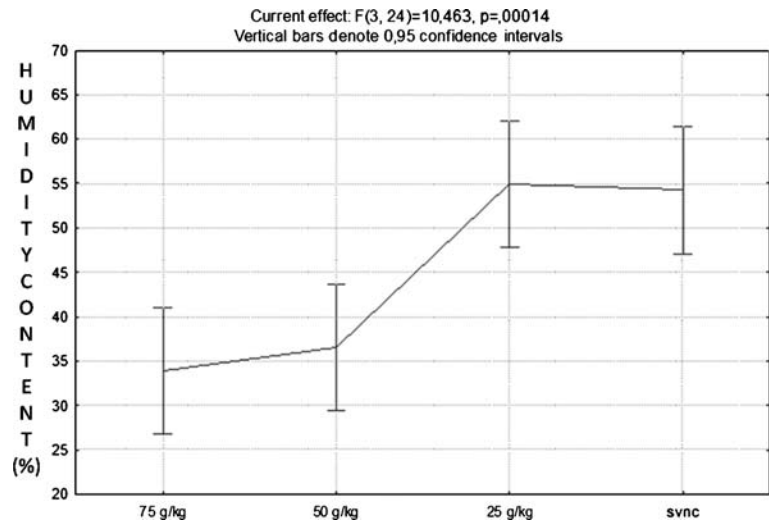
hydrocarbons which can repel water due to polarity differences. Thus, water cannot be distributed uniformly, which then may prevent water from being absorbed by plants (Merkl et al. 2004). Additionally, a correlation between number of branchings and humidity content can be done, as the plant needs to be hydrated so that its cells, tissues and organs can start the differentiation process which results in the growing and development of the plant. The increasing number of branchings is an evidence of such development and it can be noticed that individuals in contact with lower petroleum concentration contained a higher number of branchings (Fig. 3).

Samples SVNC and SVC contaminated at 25 g kg^{-1} revealed dry biomass values higher than samples contaminated at 50 g kg^{-1} and 75 g kg^{-1} (Fig. 6). Such results may be due to the fact that these samples were kept in contact with higher concentrations of the pollutant, and were therefore exposed to more toxicity caused by petroleum. According to some authors, species that are kept in contact with soils contaminated with petroleum hydrocarbons may have their biomass reduced significantly as a consequence of the stress imposed by the pollutant (Huang et al. 2005; Merkl et al. 2005; Salanitro et al. 1997; Rivera-Cruz et al. 2006).

Fig. 6 Relation between dry biomass of plants' aerial part in contact with different petroleum concentrations. Comparison between averages obtained for the dry biomass percentage analysis through variance analysis (ANOVA). *Vertical bars* indicate 0.95 of confidence intervals
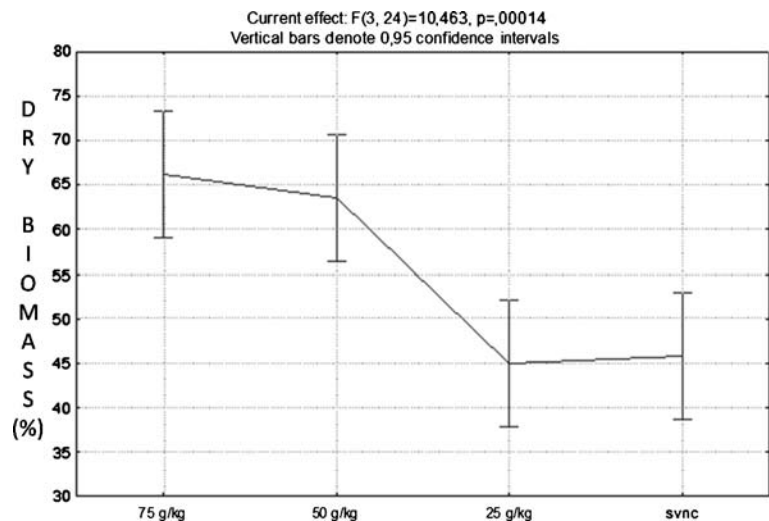
3.2 Statistics Analysis

The variance analysis (ANOVA), double factor ($p < 0.05$) revealed that dry biomass of SVNC treatment differs significantly from the other treatments SVC 25 g kg^{-1} ($p = 0.00$), SVC 50 g kg^{-1} ($p = 0.00$) and SVC 75 g kg^{-1} ($p = 0.00$;Fig. 6). In relation to the percentage of dry biomass and humidity content, SVNC differs significantly only from treatments SVC 50 g kg^{-1} ($p = 0.00$) and 75 g kg^{-1} ($p = 0.00$; Figs. 5 and 6). As for the analysis of pairs of leaves (Fig. 2) and roots volume (Fig. 4), SVNC treatment differs significantly from all other SVC treatments 25 ($p = 0.00$), 50 ($p = 0.00$) and 75 g kg^{-1} ($p = 0.00$).

3.3 Chromatographic Analysis

Zero time, 60- and 424-day samples revealed similar chromatographic profiles (Fig. 7), detecting the presence of petroleum hydrocarbons homologous series. Peaks eluting at lower retention times represent the light fractions of petroleum and peaks eluting at higher retention times represent petroleum heavy fractions, as the temperature increases during the run. GC-MS analysis revealed that the homologous series represent a sequence of alkanes with an increasing number of carbons (Table 1).

When peak areas obtained for the contaminated treatments 25, 50 and 75 g kg^{-1} were compared with the areas for peaks at the same retention times observed for freshly contaminated samples (zero time), a significant reduction of total petroleum hydrocarbon area was observed. The analysis of the 60-day samples showed a petroleum hydrocarbon area reduction higher than 60% for all compounds and at 424 days, the reduction was more than 94% for all compounds (Table 1).

Fig. 7 Chromatographic profile of zero time (a), 60 (b) and 424 days (c) soil samples
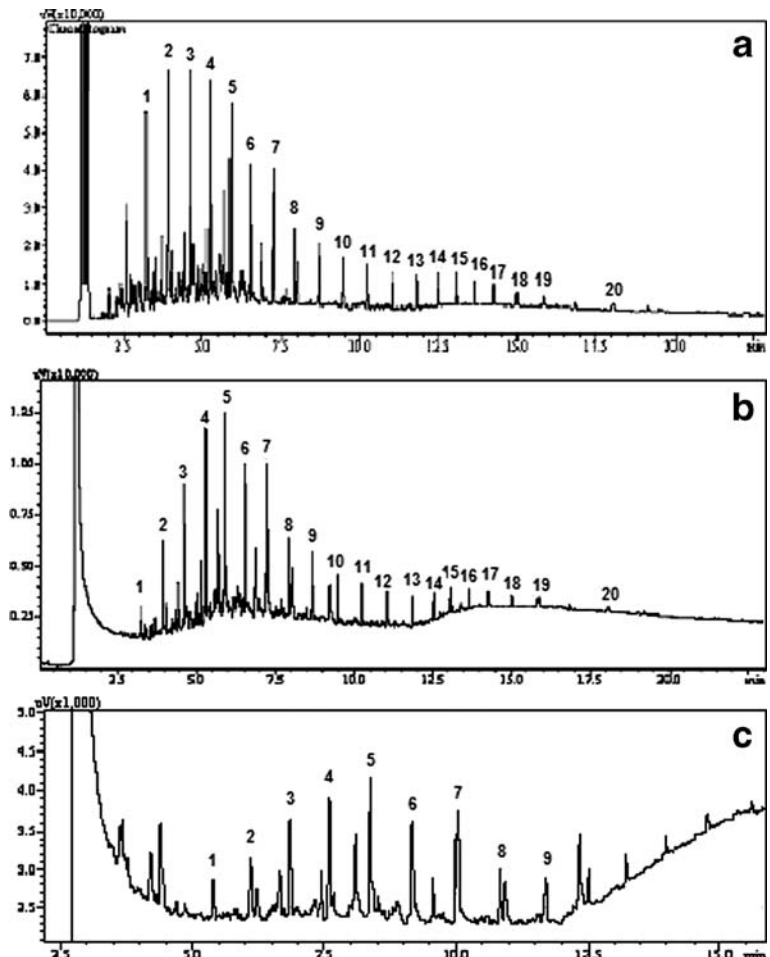
Table 1 Compounds and percentage of area reduction from samples of group SVC with 25 g kg^{-1}, 50 g kg^{-1} and 75 g kg^{-1}

Compounds	Experiment at 60 days Peak area reduction (%)			Experiment at 424 days Peak area reduction (%)		
	25 g kg^{-1}	50 g kg^{-1}	75 g kg^{-1}	25 g kg^{-1}	50 g kg^{-1}	75 g kg^{-1}
Nonane	95.7	97.23	96.57	100	99.66	99.79
Decane	92.5	93	90.92	100	99.64	99.36
Undecane	84.3	86.26	84.06	99.28	99.1	98.9
Dodecane	79.1	81.3	79.36	99.57	98.59	96.46
Dodecane 4,6-dimethyl	74	76.66	76.91	98.69	95.86	98.34
Tridecane	70.7	74.2	75.78	98.69	97.82	97.71
Dodecane 2,6,10-trimethyl	70.7	72.23	71.1	98.89	94.95	98.29
Tetradecane	68.1	73.44	75.82	99.37	97.66	98.6
Pentadecane	69.6	74.79	76.91	96.84	98.02	100
Hexadecane	72.4	78.17	79.51	100	100	100
Heptadecane	71.8	80.81	82.09	100	100	100
Octadecane	71	84.05	84.62	100	100	100
Nonadecane	71.7	86.36	88.16	100	100	100
Hexadecane 2,6,10,14-tetramethyl	71.4	88.74	87.99	100	100	100
Eicosane	78.7	93.01	94.26	100	100	100
Heptadecane 2,6,10,14-tetramethyl	65.8	86.87	85.75	100	100	100
Heneicosane	73.3	89.29	87.73	100	100	100
Docosane	82	91.48	89.61	100	100	100
Pentadecane	69.2	90.12	89.96	100	100	100
Heptacosane	57.4	83.59	88.4	100	100	100

In Table 1, it can be observed that the 60-day samples showed a higher hydrocarbon reduction area for the peaks with lower retention times, which correspond to the shorter carbon chains. This supports the supposition that such chains are more easily degraded by the rhizosphere microorganisms.

Samples from the non-vegetated but contaminated (SNVC) treatments at 60 days had area values 65% higher than those from the most contaminated vegetated treatments (SVC 75 g kg^{-1}) with the same experimental duration (result not shown), providing evidence that natural attenuation alone is not as effective as phytoremediation with *S. commersoniana*.

The 60-day samples with *S. commersoniana* showed a higher reduction of hydrocarbon peaks when compared to the 424-day samples. Such a result may represent that from 60 to 424 days, the

Table 2 Relation between petroleum concentrations and microorganisms density in samples of soil

SAMPLE	MOLDS (*CFU/g)		YEASTS (*CFU/g)		BACTERIAS (*CFU/g)	
	60 days	424 days	60 days	424 days	60 days	424 days
SVNC	1.33×10^9	1.87×10^9	$<10^9$	$<10^9$	$<10^9$	$<10^9$
SVC 25 g kg^{-1}	1.67×10^9	0.83×10^9	0.3×10^9	$<10^9$	0.67×10^9	2.0×10^9
SVC 50 g kg^{-1}	1.33×10^9	0.80×10^9	0.83×10^9	$<10^9$	1.67×10^9	2.5×10^9
SVC 75 g kg^{-1}	151.5×10^9	$<10^9$	138.5×10^9	$<10^9$	$<300 \times 10^9$	3.7×10^9

remediation process is slower than from one to 60 days. Towards the end of the experiments rates may be slower because most of the available organic compounds have already been degraded. The faster initial rates are fortunate because this reduces the time that the area will be exposed to the pollutant, and consequently, minimises negative impacts that can be caused by its presence.

3.4 Rhizosphere Microbial Analysis

Microbiological analysis of the 60-day samples revealed that soil samples of group SVC at 75 g kg^{-1} had an intensified growth of both bacteria and fungi in comparison with the concentrations of 50 and 25 g kg^{-1}. This might indicate that petroleum can stimulate the growth of microorganisms in soil by providing a carbon source that may be used in their cell synthesis (Ayotamuno et al. 2006; Table 2).

Additionally, a correlation between results from Tables 1 and 2 can be made, as the reduction of peaks percentage area is higher at higher contaminated soil and in the rhizosphere microbial analysis, microbial populations were higher at these sites, which might indicate that petroleum can stimulate their growth. So, microbial proliferation in the treatment SVC 75 g kg^{-1} could justify the fact that the percentage of reduction of hydrocarbons peaks was higher at this treatment.

The analysis of 424-day samples demonstrated a decrease in moulds density when compared to 60-day samples (Table 2). Such a decrease might have happened due to the lack of carbon sources as a reduction of petroleum hydrocarbons in the 424-day samples was noticed on the chromatograms. Also, an increase in bacteria density was detected which leads to the thought that bacteria might have inhibited fungi growth as these microorganisms compete for nutrients (Merkl et al. 2005; Rivera-Cruz et al. 2006). Additionally, it has been reported that bacteria are less susceptible to the presence of petroleum and are capable of growing and surviving in a petroleum-contaminated area (Ferrera-Cerrato et al. 2006).

Yeasts were not found in the 424-day samples. Possible reasons include availability of carbon source, yeasts sensitivities to pH changes (Rivera- Cruz et al. 2006) and the unicellular structure of yeast (vs. moulds) which may justify yeast's susceptibility (Roitman et al. 1991; Jay et al. 2005).

4 Conclusions

According to the obtained results, it can be concluded that petroleum is able to stimulate the growth of microorganisms in the rhizosphere since in SVC 75 g kg^{-1} samples, higher microbial density and diversity were found. In the 424-day samples, the number of microorganisms decreased, probably due to the lack of carbon source. Such results reveal the importance of the microorganisms' role on petroleum hydrocarbons degradation. It is believed that *S. commersoniana* might have accelerated the remediation process, through the strategies of rhizodegradation and phytostimulation, because a significant reduction of petroleum hydrocarbons peak's areas was observed after 60 days in samples planted with the tree. The use of a native species in the remediation process represents a great advantage as they are already adapted to the area conditions and, consequently, have higher chances of tolerating the presence of a pollutant and surviving in the area.

References

Ayotamuno, M. J., Kogbara, R. B., Ogaji, S. O. T., & Probert, S. B. (2006). Bioremediation of a crude-oil polluted agricultural-soil at Port Harcourt. *Applied Energy, 83*(11), 1249–1257.

Cunningham, S. D., Anderson, T. A., Schwab, A. P., & Hsu, F. C. (1996). Phytoremediation of soils contaminated with organic pollutants. *Advances in Agronomy. v., 56*, 55–114.

Davies, W. J., & Bacon, M. A. (2003). Adaptation of roots to drought. In H. de Kroon & E. J. W. Visser (Eds.), *Root ecology* (pp. 173–192). Berlin: Springer.

Dietz, A. C., & Schnoor, J. L. (2001). Advances in phytoremediation. *Environmental Health Perspectives, 109*(1), 163–168.

Escalante-Espinosa, E., et al. (2005). Improvement of the hydrocarbon phytoremediation rate by *Cyperus laxus* Lam. inoculated with a microbial consortium in a model system. *Chemosphere, Oxford, 59*(3), 405–413.

Euliss, K., et al. (2008). Greenhouse and field assessment of phytoremediation for petroleum contaminants in a riparian zone. *Bioresource Technology, Barking, 99*(6), 1–11.

Ferrera-Cerrato, R., López-Ortiz, C., Alarcón, A., Martinez-Delgadillo, J., Trejo-Aguilar, D., Mendonza-López, R., et al. (2006). Tolerance and phytoremediation of crude-oil contaminated soil by three graminoids species" (in

Spanish). 2006. In *Proceedings of the 2nd. International Meeting on Environmental Biotechnology and Engineering*, Montecillo, Postgraduate College, Department of Microbiology, Campus Montecillo, Mexico State.

Gentry, T. J., Rensing, C., & Pepper, I. L. (2004). New approaches for bioaugmentation as a remediation technology. *Critical Reviews in Environmental Science and Technology, 34*, 447–494.

Hernández-Acosta, E., Ferrera-Cerrato, R., & Almaráz-Rodríguez, J. J. (1997). Influence of hydrocarbons complex in rhizosphere communities and in frijol Michoacán growth (in Spanish). Master's Theses, Department of Edaphology? Postgraduate College, Montecillo, Mexico.

Hou, F. S. L., et al. (2001). Variations in phytoremediation performance with diesel-contaminated soil. *Environmental Technology, London, 22*(2), 215–222.

Huang, X.-D., et al. (2005). A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPH's) from soils. *Microchemical Journal, New York, 81*(1), 139–147.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern food microbiology*, 6th ed. Springer, pp. 36.

Kechavarzi, C., Pettersson, K., Leeds-Harrison, P., Ritchie, L., & Ledin, S. (2007). Root establishment of perennial ryegrass (*L. perenne*) in diesel contaminated subsurface soil layers. *Environmental Pollution, 145*(1), 68–74.

Lin, Q., & Mendelssohn, I. A. (1998). The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. *Ecological Engineering, Amsterdam, 10*(3), 263–274.

Lorenzi, H. (2002). *Brazilian trees: manual of identification and native plants culture from Brazil (In Portuguese)* (4th ed., p. 128). Nova Odessa: Instituto Plantarum de Estudos da Flora.

Maranho, L. T., Galvão, F., Preussler, K. H., Muniz, G. I. B., & Kuniyoshi, Y. S. (2006). Structural potential of *Sebastiania commersoniana* (Baill.) L. B. SM. & Downs, in phytoremediation of petroleum contaminated soils, (in Portuguese). In *Proceedings of the 2nd. International Meeting on Environmental Biotechnology and Engineering*. Montecillo, Postgraduate College, Department of Microbiology, Campus Montecillo, Mexico State, pp. 1-7, 2006.

Maranho, L. T. (2004). Effects of petroleum pollution in the structure of *Podocarpus lambertii* Klotzsc ex Endl. (Podocarpaceae) leaves, (in Portuguese). Thesis. Federal University of Parana, Curitiba.

Merkl, N., Schultze-Kraft, R., & Infante, C. (2004). Phytoremediation in the tropics: the effect of crude oil on the growth of tropical plants. *Bioremediation Journal, Boca Raton, 8*(3–4), 177–184.

Merkl, N., Schultze-Kraft, R., & Infante, C. (2005). Phytoremediation in the Tropics—influence of heavy crude oil on root morphological characteristics of graminoids. *Environmental Pollution, 138*(1), 86–91.

Michel, J., Henry, J. R., Charles, B., & Thumm, S. (2002). Shoreline assessment and environmental impacts from the M/T Westchester oil spill in the Mississippi River. *Spill Science & Technology Bulletin, Oxford, 7*(3–4), 55–161.

Palmroth, M. R. T., Pichtel, J., & Puhkka, J. (2002). Phytoremediation of subarctic soil contaminated with diesel fuel. *Bioresource Technology, Barking, 84*(3), 221–228.

Phillips, L. A., Charles, W. G., & Germida, J. J. (2006). Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbons contaminated flare-pit soil. *Soil Biology and Biochemistry, Oxford, 38*(9), 2823–2833.

Reis, J. C. (1996). *Environmental control in petroleum engineering*. Houston: Gulf.

Rivera-Cruz, M. del C., Trujillo-Narcía, A., Ferrera-Cerrato, R., Rodríguez-Vázquez R., Volke-Haller, V., Sánchez-García, P., et al. (2006). Phytoremediation of soils polluted with Benzo(a)Pyrene using indigenous microorganisms and creeping rivergrass [Echinochloa polystachya (H.B.K.) Hitchc.], (in Spanish). Universidad y Ciência, vol. 22(1), pp. 1-12.

Roitman, I., Travassos, L. R., & Azevedo, J. L. (1991). Microbiology treaty (in Portuguese). Manole, São Paulo, SP, Brazil, vol. 2, pp. 17-28.

Salanitro, J. P., et al. (1997). Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. *Environmental Science & Technology, Easton, 31*(6), 1769–1776.

Sangabriel, W., et al. (2006). Tolerancia y capacidad de fitorremediación de combustóleo en el suelo por seis especies vegetales. *Revista Internacional de Contaminación Ambiental, México, 22*(2), 63–73.

Shen, J., & Bartha, R. (1996). Metabolic efficiency and turnover of soil microbial communities in biodegradation tests. *Applied and Environmental Microbiology, [Washington], 62*(7), 2411–2415.

Solon, J. L., et al. (2005). *Banco de sementes do solo em três fases sucessionais de uma floresta estacional decidual em Santa Tereza, RS, vol 15, n. 4* (pp. 359–370). Santa Maria: Ciência Florestal.

Silva, N. da, Junqueira, V. C. A., & Silveira, N. F. de A. (2001). *Manual of food microbiology analysis methods*. (in Portuguese). 2nd. ed., Varela, São Paulo, SP, Brazil.