Appl Biochem Biotechnol (2009) 157:10–22
DOI 10.1007/s12010-009-8531-1

Phytodegradation Potential of *Erythrina crista-galli* L., Fabaceae, in Petroleum-Contaminated Soil

Vanessa de Farias · Leila Teresinha Maranho · Eliane Carvalho de Vasconcelos · Marco Aurélio da Silva Carvalho Filho · Luiz Gustavo Lacerda · Jayme Augusto Menegassi Azevedo · Ashok Pandey · Carlos Ricardo Soccol

Received: 18 December 2007 / Accepted: 20 January 2009 /
Published online: 10 March 2009

Abstract This work aimed at investigating both the tolerance and the phytodegradation potential of *Erythrina crista-galli* L. in petroleum-contaminated soil. It consisted in analyzing *E. crista-galli* germination, surviving, growth, and development when cultivated at different contaminant concentrations and pollutant degradation rates. This specimen was selected because it presented a special behavior among others also exposed to petroleum in an accident that occurred in the Araucaria region (south of Brazil), resulting in a four-million-liter oil spill. The experiment was carried out in a greenhouse containing non-contaminated soil (NCS), vegetated contaminated soil (VCS), and non-vegetated contaminated soil (NVCS) at the following petroleum concentrations: 25 g kg^{-1} (VCS-25), 50 g kg^{-1} (VCS-50), and 75 g kg^{-1} (VCS-75). After 60 days, the soil samples were

V. de Farias
Department of Biology Science, Universidade Positivo, Prof. Pedro Viriato Parigot de Souza Street, 5300, CEP 81.280-330 Curitiba, PR, Brazil

L. T. Maranho · E. C. de Vasconcelos · M. A. da Silva Carvalho Filho
Program on Environmental Management, Universidade Positivo, Prof. Pedro Viriato Parigot de Souza Street, 5300, CEP 81.280-330 Curitiba, PR, Brazil

L. T. Maranho · J. A. M. Azevedo
Environmental Biotechnology, Department of Chemical Engineering, Universidade Federal do Paraná, Av. Cel. Francisco H. dos Santos, 210, Jardim das Américas, Curitiba, PR, Brazil

A. Pandey
National Institute for Interdisciplinary Science & Technology (NIST) (Formerly Regional Research Laboratory), CSIR, Trivandrum 695019, India

L. G. Lacerda · J. A. M. Azevedo · C. R. Soccol (✉)
Biotechnology Process, Department of Chemical Engineering, Universidade Federal do Paraná, Av. Cel. Francisco H. dos Santos, 210, Jardim das Américas, Curitiba, PR, Brazil
e-mail: soccol@ufpr.br

Humana Press

analyzed by gas chromatography. Germination was more and more evident as higher petroleum concentrations were observed. The surviving rates of groups NCS, VCS-25, VCS-50, and VCS-75 were 64%, 70%, 61%, and 96%, respectively. The VCS group growth was reduced when compared to the control group (NCS). The individuals exposed to petroleum pollution presented differences in the anatomic structure of their roots when compared to the NCS group. It was observed that the petroleum degradation rate was higher for VCS group than for NVCS. *E. crista-galli* is potentially recommended for petroleum-contaminated soils because of its positive association in the presence of contamination.

Keywords Phytodegradation · Petroleum · *Erythrina* · Root · Fabaceae

Introduction

The techniques used to clean up contaminated areas strongly depend on the type of contaminant, bioavailability, and soil properties [1]. Furthermore, the processes must aim at efficiency, simplicity, and feasibility [2, 3]. Phytoremediation is emerging as a promising technology for remediating contaminated soils [3, 4].

Through phytoremediation, soils contaminated with organic and inorganic substances as well as heavy metals, petroleum, toxics from agricultural processes, and many residues from industries can be recovered, eliminated, or even transformed into less toxic products for the environment [5].

The phytoremediation process consists in the use of plants, rhizosphere-associated organisms, and soil amendments [6]. When compared to traditional techniques—such as pumping and treatment and physical removal of contaminated layer—phytoremediation has been considered advantageous especially because of its decontamination efficiency and low costs [5, 7].

When used to remove petroleum contaminants, phytoremediation has been proven to be a good alternative especially in tropical regions where the climate helps in the development of plants and in their microbiological activity [3, 4].

Michel et al. [8] affirm that petroleum constitutes a pollutant that can persist in the environment for a long period until vegetation recovers completely, and its persistence can be explained by the slow biodegradation of hydrocarbons. The petroleum action on the plants can be direct when there is contact with oil and indirect when there are biotic and abiotic alterations associated to plant development [2]. The direct contact between plant and contamination through the vascular tissues modifies the solubility and permeability of the cell membranes, decreases the gaseous exchanges, promotes chlorosis, and inhibits seed germination [2, 3, 9]. Moreover, direct contact modifies physical conditions and soil fertility [3, 9].

Although the aromatic hydrocarbons are degraded, the rate of this process decreases as the availability of oxygen in the environment decreases. Pezeshki et al. [10] have observed an increase in the intercellular spaces and a larger diameter of the root cortical cells of *Spartina patens* (Aiton) Muhl. in petroleum-contaminated soil and attributed this response to the low redox potential of the soil. The presence of roots increases microorganism populations, improves the physical structure of the soil, and enhances hydrocarbon degradation [11].

Phytoremediation in petroleum-contaminated soils occur in an indirect form, since the root development allows oxygen diffusion to the sediment where there are microorganisms that can effectively make use of aerobic breathing.

Several species of Poaceae, Fabaceae, Mimosaceae, and Caesalpiniacae have been studied because of their contaminant degradation potential. Studies report that degradation of petroleum and its derivatives in soils vegetated with different species of those families, such as *Festuca arundinacea* Vill., *Sorghum bicolor* L. Moench, *Vigna sinensis* (L.) Endl. ex Hassk., *Medicago sativa* L., *Juncus roemerianus* Scheele, *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf., and *Panicum maximum* Jacq., have been greater when compared to soils with no vegetation on [3, 9].

Much importance is given to plants of the Poaceae species due to the fact that they favor the reduction of contaminants [12–15]. Plants and their roots influence directly contaminant degradation through a change in the physical and chemical conditions of the soil [5]. The potential of vegetable species, such as phytoremediators, in petroleum-contaminated soils is often due not to the absorption of compounds but to their development, which allows for an improvement in the soil condition [16].

The selection of plants belonging to the group generically known as legumes, to which the *Erythrina crista-galli* L. belongs, can promote the cleaning up of petroleum-contaminated areas, since these plants are associated—in their roots—to nitrogen-fixing bacteria. These plants are selected for phytoremediation because they can fix nitrogen and do not compete with other microorganisms and plants [9]. The nitrogen-fixing bacteria can be an option for the treatment of contaminated soil by petroleum compounds, and its application influences directly the incorporation of nitrogen in soils where there is an excess of carbon [17].

In order to be a potential phytoremediation promoter, the species must the evaluated in its tolerance in the presence of several contamination concentrations as well as in its capacity to decontaminate soil [3, 4]. There are only few studies related to the phytoremediation potential of petroleum-contaminated soils, so further researches are necessary to evaluate this technique, especially those using native species.

This is the reason why *E. crista-galli* L., Fabaceae, was chosen for the present work. This species, also known as "corticeira-do-banhado", is a 6- to 10-m tall native tree from the forests in the south region of Brazil, which have survived after an accidental four-million-liter petroleum spill when most other species were killed. It is very important in landscape gardening for it is very decorative when in bloom. The present research aimed at observing and evaluating seed germination, plant survival, and the development of *E. crista-galli* in different petroleum concentrations and contaminant degradation rates.

Material and Methods

Seeds from eight *E. crista-galli* L. plants were obtained in a subtropical forest fragment in southern Brazil. The soil used in the experiments was obtained in a contamination-free area next to the coordinate axes 25°34′02.5″ S, 49°20′53.5″ W at 910 m above sea level. The soil is characterized by presenting a ph 6.5 as well as 26.9 g dm^{-3} and a clayish texture. There, four contaminated substrates were prepared containing different Brazilian petroleum concentrations: non-contaminated soil (NCS), contaminated soil (VCS) with (milligrams petroleum per kilogram of dry soil) 25,000 mg kg^{-1} (VCS-25), 50,000 mg kg^{-1} (VCS-50), and 75,000 mg kg^{-1} (VCS-75). Additionally, treatments with non-vegetated contaminated soil were prepared in the same concentrations to verify the natural degradation of petroleum consisting of the non-vegetated-contaminated soil groups, NVCS-25, NVCS-50, and NVCS-75, with six repetitions each [3, 4, 36].

The experiments were carried out in a greenhouse under temperature between 25°°C and 30°°C and relative humidity between 85% and 90%. The contaminated (VCS) and non-

contaminated (NCS) soils were inserted in 22-cm-tall and 24-cm diameter pots. Each pot received one seed of *E. crista-galli* and was thereafter irrigated daily.

A method proposed by Sangrabiel et al. [9] was used for analyzing the germination process. Each treatment received 25 viable seeds, which were submitted to the standard germination test [18] and observed daily. The starting point of germination was understood to be the point when tegument protrusion occurs. After data collection, the percentage of germination was estimated according to $\%G=(\text{ni}\ N^{-1})\times 100$, where ni corresponds to the total number of germinated seeds and N^{-1} corresponds to the total number of seeds planted for germination.

The experiment was controlled during 120 days and all factors pertinent to the aims were observed. After 60 and 90 days from the beginning of germination, the roots were removed from the soil and washed. Subsequently, they were evaluated on their growth, stem and root length, and number of nodules [3]. The Student's *t* test was used for comparing the treatments. The differences were considered significant in cases in which $p\leq 0.05$.

The anatomic analyses were carried out on sections of the main and lateral roots, located at 3 cm from the apex, with six repetitions for treatment. The samples were collected and fixed in FAA 70 for 48 h [19] and subsequently kept in 70% ethanol until final processing [20].

For obtaining the permanent slides, samples were selected from the main and secondary roots located at 2 cm from the apex. These samples were involved in glycol methacrylate (JB-4) following the procedure described by Feder and O'brien [21] and the manufacturer's recommendations (Polysciences Inc.). The sections were done in a rotation microtom (Leica RM2125). They were 7 μm thick and were later stained with 1% toluidine blue [22]. The slides were assembled with synthetic resin (Entelan®).

The description of the cross-cut sections was carried out using a photonic microscope (Olympus—CX41RF). A photomycroscope (Olympus—BX 41) with image caption was used for obtaining the illustrations, and the scales were obtained under the same conditions as those of the photos.

The petroleum degradation was determined by extracting and quantifying petroleum in treatments with (VCS-75) and without (NVCS-75) vegetation on the 60th day. The extracting process was carried out using dichloromethane as solvent under agitation [3, 23]. Subsequently, 2 g of sodium sulfate (Na_2SO_4), 2 g of each soil sample, and 5 mL of dichloromethane (CH_2Cl_2) were added in a more powerful filter. Solutions were kept under agitation for 1 h at 3,000 rpm. After agitation, samples were centrifuged at 2,500 rpm for 15 min. The supernatant was extracted using a Pasteur pipe and stored in amber glasses. Samples were extracted more than twice and the supernatants were collected.

All extracted samples were analyzed by a Shimadzu GC-2010 gas chromatograph. The DB-5 column had a 0.25-mm inside diameter and a 30-m-long, 0.25-μm-thick film. The carrier gas (hydrogen; 1.0 mL min^{-1}) and nitrogen were used as a makeup gas. The initial oven temperature was maintained at 70 °C for 4 min then increased to 190°°C (20°°C min^{-1}), to 250°°C (10 min^{-1}), and finally to 280°°C (30°°C min^{-1}) and kept for 10 min. The temperature of the injector port was 250°°C and the detector temperature was 280°°C. The injection volume was 0.5 μL.

Results and Discussion

The petroleum-derived hydrocarbons have a negative influence concerning plant growth since this behavior is observed in several researches [3, 4, 9, 24–26]. After the soil is contaminated with petroleum, its physical, chemical, and biological conditions are altered.

The hydrophobic characteristic of petroleum prevents the water from spreading in the soil, resulting in a lack of water in the environment. However, some plants can adapt to the lack of water by increasing root diameter [4].

The germination occurred in 3 days in the following groups: VCS-25 and VCS-75. It had been observed that the group containing the largest concentration of contaminants (VCS-75) showed a germination rate of 44%, whereas other petroleum treatments and controls showed an initial germination rate of 20% during the first 3 days.

The final germination rate analysis (Table 1) demonstrated that treatment related to group VCS-75 had the best result: its germination rate was 92%, whereas groups NCS, VCS-25, and VCS-50 showed germination rates of 88%, 80%, and 76%, respectively (Table 1). The data suggest that despite the treatment adopted, the *E. crista-galli* seeds showed a good germination response to contamination. Other experiments have showed that corn (*Zea mays* L.) germination in soil contaminated with crude oil is affected by the pollutant [27].

The presence of hydrocarbons even at low concentrations, between 4000 and 8,000 mg kg^{-1}, can be severely negative for germination and consequently reduce the emergence of new species in the polluted environment [28].

Aromatic compounds can decrease the seed production rates, even inhibiting their embryonic development [2, 3]. In addition, direct contact between the plant and the contaminant can change both the cellular membrane permeability and solubility. However, it has been observed that some plants can survive after its exposition to the pollutant. The same plant population response in the presence of the pollutant is a function of age and even of season [10].

Data obtained demonstrate that during the experiments, *E. crista-galli* seeds presented positive behavior in the presence of contamination, against what the previous literature states [3, 5, 10, 29]. Several authors have reported that petroleum can positively affect the seed germination of some species. In these cases, the petroleum fractions can work as auxins that help in the germination process [30, 31].

The plants selected for phytoremediation must have the ability to adapt to the pollutant concentrations as well as to the environmental conditions [2, 3]. The plant exposition to petroleum can increase or decrease the biomass or not produce phytotoxics effects [31]. This response is a function of soil and petroleum types, species, and general experimental conditions.

In relation to plant survival, it was observed that after having initiated the germination process, many seeds suffered a side development of fungi affecting further seedlings development. The highest survival rate was related to the VCS-75 group, corresponding to 96% (Table 1), whereas the control and the VCS-25 and VCS-50 groups showed a surviving rate of 64%, 70%, and 61%, respectively. The highest value can be related to the less quantities of fungi probably due to petroleum toxicity. There are a few legumes showing resistance to hydrocarbons [9]. The same authors, studying *Clitoria ternatea* L.,

Table 1 Germination and survival rate of *E. crista-galli* L. in different petroleum concentrations.

Treatment	Germination rate (%)	Survival rate (%)
NCS	88	64
VCS-25	80	70
VCS-50	76	61
VCS-75	92	96

Phaseolus coccineus Lam., and *Cicer arietinum* L., have observed that only *P. coccineus* resisted to the contaminant and that other species died after 42 and 56 days, respectively. It was also observed that the *P. coccineus* surviving rate was 50%, and the average height of the plants was lower due to pollutant exposition.

Other studies concerning phytoremediation have been conducted using different types of grass as decontamination agents [3, 9]. However, when compared to legumes, grass shows lesser biomass, its root system cannot attain great depths, and it cannot fix atmospheric nitrogen.

After 60 days, in the control group, the plant was 25 cm tall (100%; Table 2), whereas VCS-25, VCS-50, and VCS-75 groups were 20.82 cm (83%), 13.17 cm (53%), and 22.72 cm (91%) tall, respectively. After 90 days, the results increased as follows: control group showed 19.75 cm (100%); VCS-25, VCS-50, and VCS-75 showed 13.83 cm (70%), 12.92 cm (65%), and 17.75 cm (90%), respectively. A significant difference was detected between treatments NCS and VCS-25 (p=0.00), VCS-25 and VCS-50 (p=0.00), and VCS-50 and VCS-75 (0.00) on the 60th day of the experiment, and on the 90th day, between NCS and VCS-50 (p=0.02), VCS-25 and VCS-50 (p=0.05), and VCS-50 and VCS-75 (0.00). Several plants that exhibit decreasing growth are suffocated by the crude oil and by the lack of necessary oxygen for their metabolic processes [27]. The same authors observed that hydrocarbon degradation by the action of bioremediation leads to an increasing Mn toxicity and availability. Besides, the smallest values in plant tallness recorded on the 90th day of the experiment reflect a natural behavior of the *E. crista-galli*, since we are dealing with a deciduous species which lost all its leaves in late winter and started growing again in the following period.

During hydrocarbon degradation, promoted by rhizosphere-associated microorganisms, there is a substantial consumption of nutrients. Thus, plants and microorganisms compete for these elements [4].

The aerial vegetative growth of *E. crista-galli* varied among the different treatments. As shown in Fig. 1, the control group showed better growth results than the other groups during the whole investigation.

The root growth for groups NCS and VCS-75 seemed very similar in both cases, as it could be observed after 60 and 90 days (Table 2). Also, after 90 days, all groups showed almost the same root development (Fig. 2). In the control group, after 60 days, the root volume was 2.67 mL (100%), whereas VCS-25, VCS-50, and VCS-75 groups showed

Table 2 Plant height and root system of *E. crista-galli* L. as a function of treatment and time.

Treatment	Time (days)	Plant height (cm)	Root system		
			Length (cm)	Volume (mL)	Nodules
NCS	60	25.00 ± 6.14^{a}	21.70 ± 7.68^{a}	2.67 ± 0.27^{a}	0
	90	19.75 ± 6.00^{a}	15.53 ± 4.12^{a}	2.70 ± 1.54^{a}	2.7
VCS-25	60	20.82 ± 2.92^{b}	$11.93\pm2.25^{a,b}$	2.48 ± 0.50^{b}	2.0
	90	13.83 ± 4.11^{b}	14.08 ± 5.31^{b}	2.17 ± 0.10^{b}	3.7
VCS-50	60	$13.17\pm3.45^{a,b,c}$	$10.40\pm6.28^{a,c}$	$1.30\pm0.39^{a,c}$	2.7
	90	$12.92\pm0.34^{a,c}$	17.35 ± 4.54^{c}	$1.75\pm0.45^{b,c}$	0.3
VCS-75	60	$22.72\pm2.52^{c,d}$	20.30 ± 9.74^{d}	$2.02\pm0.37^{a,c,d}$	0.7
	90	$17.75\pm1.37^{b,c,d}$	16.45 ± 5.89^{d}	$2.68\pm0.49^{b,c,d}$	0.7

Values followed by different letters are significantly different at 5% probability level ($p\leq0.05$) by Student's t test

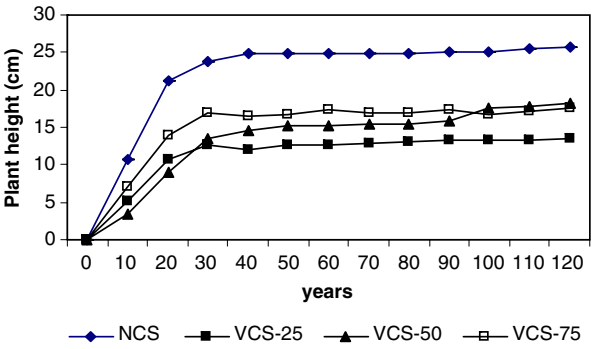

Fig. 1 Dynamics of stalk growth of *E. crista-galli* L. in different treatments with petroleum

2.48 mL (93%), 1.30 mL (48%), and 2.02 mL (75%), respectively. After 90 days, the results increased as follows: control group 2.70 mL (100%) and VCS-25, VCS-50, and VCS-75 showed 2.17 mL (80%), 1.75 mL (65%), and 2.68 mL (99%), respectively. A significant difference was detected among treatments NCS and VCS-75 (p=0.00), VCS-25 and VCS-50 (p=0.00), and VCS-50 and VCS-75 (0.01) on the 60th day, and on the 90th day, between VCS-25 and VCS-50 (p=0.05), VCS-25 and VCS-75 (p=0.03), and VCS-50 and VCS-75 (0.01). In what concerns root length, a significant difference was detected only on the 60th day of the experiment and at the moment the comparison between treatments NCS and VCS-75 (p=0.02) and NCS and VCS-50 was made, for VCS-25 and VCS-50 treatments presented average root lengths lower than those detected in the NCS treatment (Table 2).

Morphological alterations of roots can affect the degradation promoted by micro-organisms. Root alterations of *B. brizantha* (Hochst. ex A. Rich.) Stapf. and *Cyperus aggregatus* (Willd.) Endl. can help in petroleum degradation [4].

Numerous authors relate height reduction, root volume, root and aerial dry weight of various species to soil contamination by petroleum derivatives [3, 9, 31, 32].

Those features were observed for *P. maximum* Jacq., *B. brizantha* (Hochst. ex A. Rich.) Stapf., and *P. coccineus* Lam. In addition, the root system volume of *B. brizantha* increased when compared to controls [3, 9].

Root anatomy, observed in different groups, has revealed changes between individuals of the NCS group in relation to the remaining groups. It was observed that some individuals exposed to petroleum—when compared to plants in the control group (Fig. 3)—present

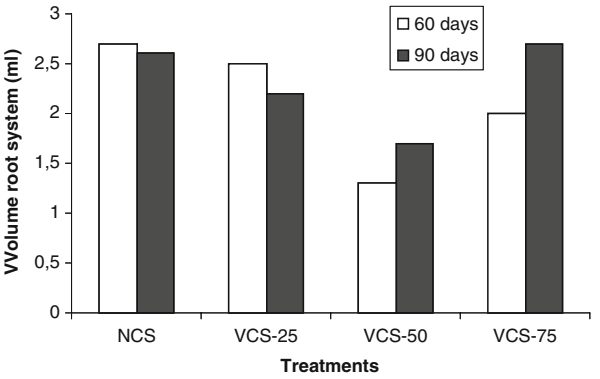

Fig. 2 Root volume of *E. crista-galli* at 69 and 90 days after being exposed to petroleum

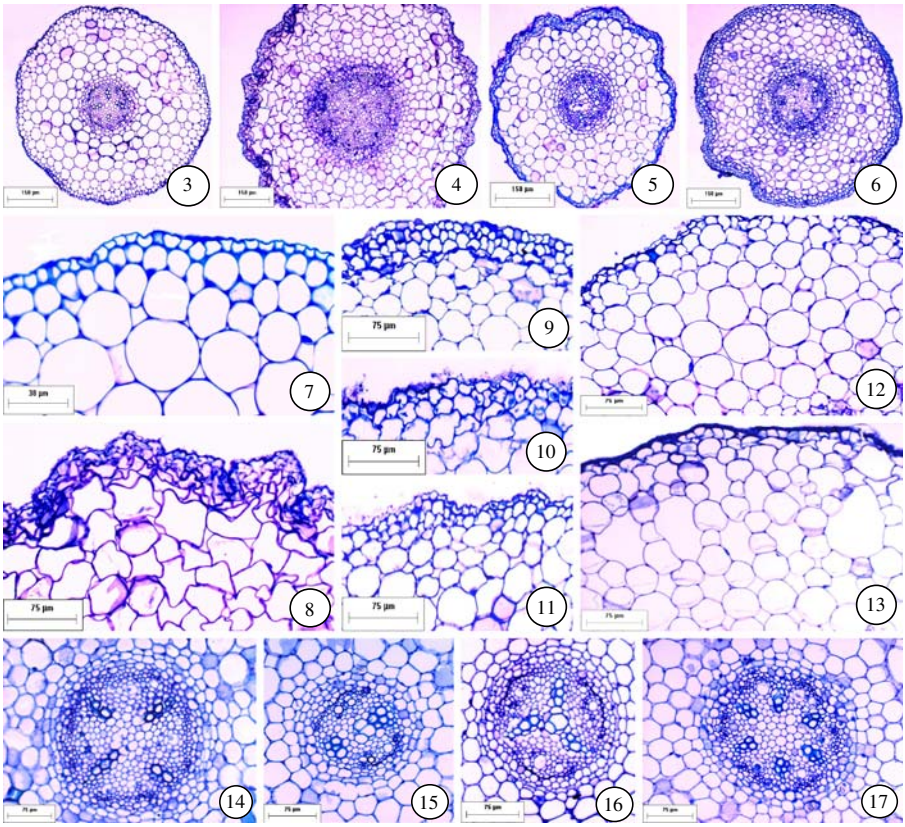

Fig. 3 *E. crista-galli* L. root: Individual NCS
Fig. 4 *E. crista-galli* L. root: General view of roots exposed to petroleum. VCS-25
Fig. 5 *E. crista-galli* L. root: General view of roots exposed to petroleum. VCS-50
Fig. 6 *E. crista-galli* L. root: General view of roots exposed to petroleum.VCS-75
Fig. 7 *E. crista-galli* L. root: Close look at the epidermis of a control's cortex
Fig. 8 *E. crista-galli* L. root: Epidermis and subepidermic layers in VCS-25
Fig. 9 *E. crista-galli* L. root: Sub-epidermic layer in VCS-75
Fig. 10 *E. crista-galli* L. root: Undulations and irregular cells
Fig. 11 *E. crista-galli* L. root: Undulations in VCS-75
Fig. 12 *E. crista-galli* L. root: Close look at the intercellular spaces
Fig. 13 *E. crista-galli* L. root: Large intercellular spaces in VCS-50
Fig. 14 *E. crista-galli* L. root: Close look at the vascular cylinder. Tetrarc
Fig. 15 *E. crista-galli* L. root: Close look at the vascular cylinder. Diarc
Fig. 16 *E. crista-galli* L. root: Close look at the vascular cylinder. Triarc
Fig. 17 *E. crista-galli* L. root: Close look at the vascular cylinder. Polyarc with six xylem poles

structural differences in the form and arrangement of the organ (Figs. 4, 5, and 6). Such alterations correspond to the organization of the epidermis (Figs. 7, 8, 9, 10, and 11) as well as to the shape and compaction of the cells that constitute the cortex (Figs. 7, 8, 12, and 13).

Among the roots collected on the 60th day of development, the control individuals showed a uniserial epidermis (Fig. 7), having circular and rectangular shape cells besides a subepidermic layer consisting of only one stratrum (Fig. 7). Groups VCS-25, VCS-50, and VCS-75, however, show a subepidermic layer formed by one to three cellular strata (Figs. 8 and 9). The individuals exposed to petroleum for 90 days, besides exhibiting a

subepidermic layer consisting of one to three strata (Fig. 10), revealed a stronger tendency to develop undulations in the epidermis of the primary root (Fig. 11).

The cortex of the individual under NCS treatment presented isodiametrical cells (Fig. 7) with small to medium intercellular spaces (Fig. 12). On the 60th day, however, it was observed that some groups tended to present an irregular form of the cells that constitute the cortex (Fig. 8). Such characteristic was observed in individuals in VCS-75. Furthermore, individuals under treatment VCS-50 had a tendency to have larger intercellular spaces (Fig. 13). An increase in those spaces can facilitate petroleum aerobic metabolism, since the element that increases the oxygen content in the soil is increased. Oxygen spreads from the roots to the rhizosphere [33], and plants with well-developed roots and wide intercellular spaces favor the liberation of oxygen into the soil [34, 35].

The vascular cylinder presented, in all treatments, a strong variation of the stele structure (Figs. 14, 15, 16, and 17), which assumed a diarc, triarc, tetrarc, and polyarc arrangement with five and six xylem poles. On the 60th day, the control individuals presented a tetrarc (Fig. 14), polyarc, and triarc (Fig. 16) conformation, but on the 90th day, the vascular cylinder structure changed in groups VCS-25 and VCS-75, which presented polyarc and diarc structure, respectively (Figs. 17 and 15).

The hydrophobic nature of petroleum keeps water from spreading homogeneously throughout the soil, and consequently, a hydric deficiency takes place in the environment [4, 35]. The plant, at its turn, adapts to the hydric deficiency present in the environment by increasing its root diameter and simultaneously reducing its growth due to its decreased permeability in dry soil [36].

Changes in the morphology and in the anatomy of roots can favor an increase in petroleum degradation [4, 36]. These authors have observed changes in the roots of *B. brizantha* (Hochst. ex A. Rich.) Stapf. and *C. aggregatus* (Willd.) Endl. as well as an increase in petroleum degradation. Numerous authors report an increase in petroleum degradation in soils under *F. arundinacea* Schreb., *S. bicolor* (Nees) Kuntze, *V. sinensis* Endl., *M. sativa* Urb., *J. roemerianus* Scheele, and *B. brizantha* (Hochst. ex A. Rich.) Stapf. when compared to non-vegetated contaminated soils [3, 4, 9, 36, 37]. However, there are few studies using native species [3, 4] for decontaminating soil polluted with petroleum, and even those existing studies represent a small portion of species that show a phytoremediation potential.

Nodules were found in all groups treated with petroleum as well as in the control group 90 days after the beginning of germination (Table 2). The VCS-75 group presented fewer nodules when compared to the other groups after 60 days and after 90 days. The same observation was made for VCS-50 and VCS-75 groups. The nodule quantities in roots exposed to petroleum tend to decrease as the oil concentration increases. Authors have reported that nodules in *Mimosa pigra* L. only appear at the following petroleum concentrations: 2791, 9035, and above 79,000 mg kg^{-1} plants do not develop nodules [31]. *Crotalaria* Scop., *Leucaena* Benth., and *Mimosa pudica* Mill. do not develop nodules at petroleum concentrations higher than 50,000 mg kg^{-1} [25].

A semi-quantitative chromatographic analysis revealed higher petroleum degradation in the VCS-75 group (Table 3 and Fig. 18) in relation to non-vegetated treatment NVCS-75 (Table 3 and Fig. 19). It was observed that a reduction of lower molecular weight chains showed higher degradation of the pollutant. Preliminary results showed that *E. crista-galli* is a promising species on which further studies are required. The analysis of percentage reduction of petroleum compounds, using gas chromatography, allowed an excellent evaluation of chemical alterations in soil (Table 3 and Figs. 18 and 19). Numerous authors report a higher degradation of petroleum contaminants in soil vegetated with *F.*

Table 3 Petroleum degradation of vegetated contaminated soil in treatment VCS-75 and non-vegetated contaminated soil in treatment NVCS-75.

Peak	Retention time (min)	Treatment		% Reduction area
		VCS-75	NVCS-75	
1	3.253	3.100	16.929	82
2	3.934	5.398	21.303	75
3	4.618	5.805	17.185	66
4	5.134	5.514	12.212	55
5	5.282	8.058	21.625	63
6	5.684	7.815	22.368	65
7	6.553	4.422	10.725	59
8	6.882	4.580	10.055	54
9	7.272	11.524	24.784	54
10	8.017	4.112	8.852	54
11	8.639	1.096	2.387	54
12	9.221	1.673	3.260	49
13	9.461	2.026	5.651	64
14	10.252	1.549	4.729	67
15	11.050	1.271	4.274	70
16	11.843	1.049	3.802	72
17	12.540	0.643	3.254	80
18	13.094	0.859	3.869	78
19	13.660	1.036	3.254	68
20	14.289	0.623	2.690	77

arundinacea Vill., *S. bicolor* L. Moench, *V. sinensis* (L.) Endl. ex Hassk., *M. sativa* L., *J. roemerianus* Scheele, and *B. brizantha* (Hochst. ex A. Rich.) Stapf. when compared to non-vegetated soil [3–5, 9, 36, 37].

Vegetated soils have increased the degradation of pentachlorophenol, pyrene, anthracene, petroleum aromatic hydrocarbon, and petroleum total hydrocarbons [12, 37–41].

The different processes involved in phytoremediation involve the morpho-physiological characteristics of the species and are different from species to species. Many attempts have

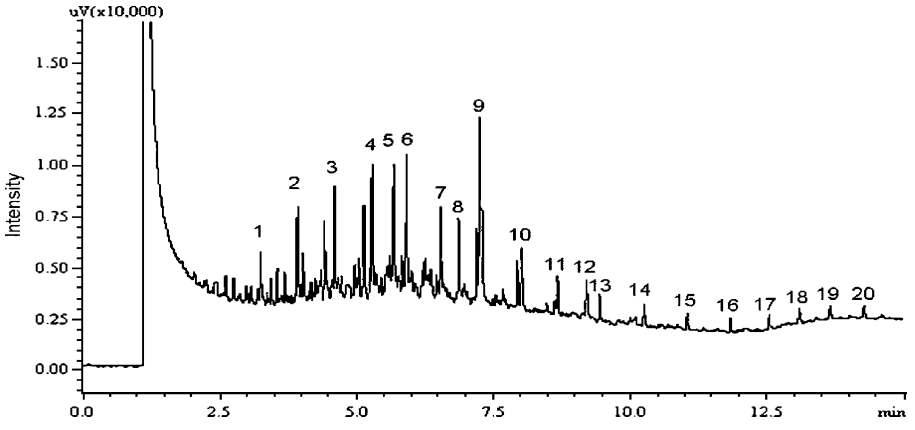

Fig. 18 Gaseous Chromatogram of vegetated contaminated soil (VCS-75) on the 90th day. Peaks *1*, *2*, *3*, *4*, and *9* show an increased degradation of petroleum compounds

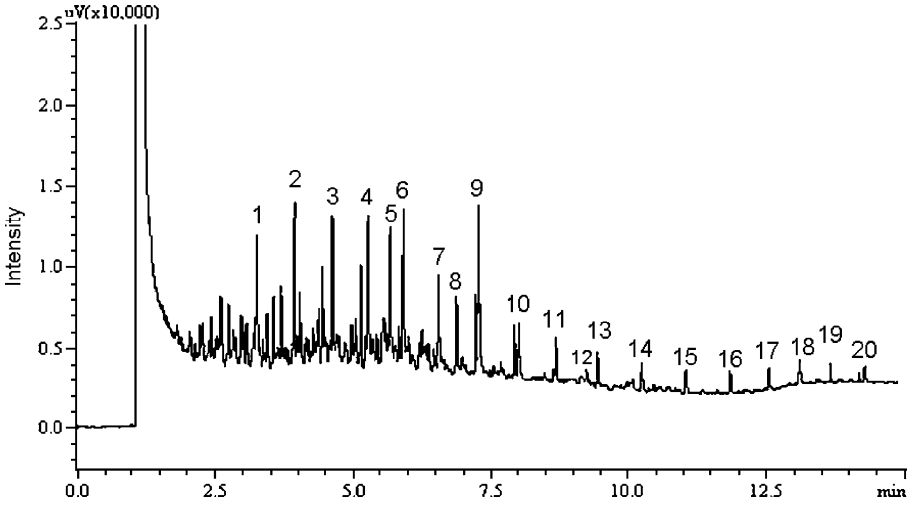

Fig. 19 Gaseous chromatogram of non-vegetated contaminated soil (NVCS-75) on the 90th day. Peaks *1*, *2*, *3*, *4*, and *9* show a decreased degradation of petroleum compounds

been made to determine some functional attributes of those plants. Generally speaking, those belonging to the legume group, such as *E. crista-galli* L., abound soils contaminated with petroleum in Europe, and this is probably so because of its ability to fix nitrogen [42]. The property of plants that favor the remediation of petroleum-contaminated soils are the following: rapid growth, high competitivity, tolerance to pollution, high nutrient absorption capacity, high translocation rate, and a large storage of reserve substances [5, 43–45]. One of the ways of identifying the phytoremediating potential species is the observation of plants that colonize contaminated areas [46]. This was done for *E. crista-galli* L., and it proved to have a remarkable presence in that soil where four million liters of petroleum was accidentally spilled.

Studies carried out in the USA and in Canada identified two hydrocarbon-tolerant species: *Helianthus annuus* L. [46] and *Amelanchier alnifolia* (Nutt.) Nutt. [47]. Those works show that both species are tolerant to hydrocarbons in the soil. Once one species has tolerance to the contaminant, it is necessary to test its ability to increase petroleum degradation [48]. Generally speaking, phytodegradation is higher in plants that have: (a) a dense root system and a large superficial area; (b) high production of enzymes used in degradation; and (c) large production of exuded substances [49, 50].

As nowadays we believe that a large portion of hydrocarbons are degraded in the rhizosphere, plants with a dense and branched root system and that do not change significantly its root volume even under stressful conditions are more adequate for phytodegradation due to their increase of rhizosphere area, resulting in increased places for the metabolic activity of microorganisms to happen [12].

The phytoremediation mechanism is not completely known. Little is known about plant–microorganism interactions, about the processes that occur in the rhizosphere, absorption, translocation, and chelating agents involved in transport and storage. Another aspect that requires further specification involves the movement of pollutants in the soil–water–plant system [50], and moreover, there is little information available about the degree of tolerance of species growing in soil contaminated with hydrocarbon.

Conclusion

The results obtained in the present study allow us to state that *E. crista-galli* L. proves to be tolerant to petroleum contamination concerning seed germination and plant growth. Furthermore, morphological modifications allow it to survive under different pollutant concentrations. For these reasons, we suggest that this plant is a potential phytodegradation promoter in petroleum-contaminated soils.

References

1. Cunningham, S. D., & Ow, D. W. (1996). *Plant Physiology*, *110*, 715–719.
2. Maranho, L. T, Azevedo, J. A. M., Preusseler, K. H., & Filho, M. A. S. (2006). Environmental biotechnology and engineering. *Proceedings of the Second International Meeting on Environmental Biotechnology End Engineering (2IMEBE)*. México City. México.
3. Ferrera-Cerrato, R., Lópes-Ortiz, C., Alarcón, A., Delgadillo-Mártinez, J., Trejo-Aguilar, D., Mendoza-López, R., et al. (2006). Environmental biotechnology and engineering. *Proceedings of the Second International Meeting on Environmental Biotechnology End Engineering (2IMEBE)*. México City. México.
4. Merkl, N., Schultze-Kraft, R., & Infante, C. (2005). *Environmental Pollution*, *138*, 86–91.
5. Cunningham, S. D., Anderson, T. A., & Schwab, A. P. (1996). *Advances in Agronomy*, *56*, 55–114.
6. Pires, F. R., Souza, C. M., Silva, A. A., Procópio, S. O., & Ferreira, L. R. (2003). *Revista Planta Daninha*, *21*(2), 335–341.
7. Perkovich, B. S., Anderson, T. A., & Coats, J. R. (1996). *Pesticide Science*, *46*, 391–396.
8. Michel, J., Henry, C. B. Jr., & Thumm, S. (2002). *Spill Science & Technology Bulletin*, *7*, 155–161.
9. Sangabriel, W., Ferrera-Cerrato, R., Trejo-Aguilar, D., Mendonza-López, M. R., Cruz-Sánches, J. S., López-Ortiz, C., et al. (2006). *Internacional de Contaminación Ambiental*, *22*, 63–73.
10. Pezeshki, S. R., Hester, M. W., Lin, Q., & Nyman, J. A. (2000). *Environmental Pollution*, *108*, 129–139.
11. Tischer, R., & Hübner, T. (2002). *International Journal of Phytoremediation*, *4*, 197–203.
12. Aprill, W., & Sims, R. C. (1990). *Chemosphere*, *20*, 253–265.
13. Qiu, X., Leland, T. W., Shah, S. I., Sorensen, D. L., & Kendall, E. W. (1997). *Phytoremediation of Soil and Water Contaminants*, *664*, 186–199.
14. Gunther, T., Dornberger, U., & Fritsche, W. (1996). *Chemosphere*, *3*, *3*, 203–215.
15. Reilley, K. A., Banks, M. K., & Schwab, A. P. (1996). *Journal of Environmental Quality*, *25*, 212–219.
16. Brandt, R., Merk, N., Schultze-Kraft, R., Infante, C., & Broll, G. (2006). *International Journal of Phytoremediation*, *8*, 273–284.
17. Hernández-Acosta, E., Ferrera-Cerrato, R., & Rodríguez, R. V. (2003). *Terra*, *21*, 81–89.
18. Ministério da Agricultura. (1992). Brasil. Regras para análise de sementes.
19. Johansen, D. A. (1940). *Plant microtechnique*. New York: McGraw Hill Book.
20. Berlyn, G. P., & Miksche, J. P. (1976). *Botanical microtechnique and cytochemistry*. Iowa: Iowa University.
21. Felder, N., & O'brien, T. P. (1968). *American Journal of Botany*, *55*, 123–142.
22. Sakai, W. S. (1973). *Stain Technology*, *48*, 247–249.
23. Schwab, A. P., Su, J., Wetzel, S., Pekerak, S., & Banks, M. K. (1999). *Environmental Science & Technology*, *33*, 1940–1945.
24. Kuhn, W., Ganbino, R., Al-Awadhi, N., Balba, M. T., & Dragun, J. (1998). *Journal of Soil Contamination*, *7*, 801–806.
25. Rivera-Cruz, M. C., Trujillo-Nárcia, A., Cruz, M. A. M., & Chávez, E. M. (2005). *Interciencia*, *30*, 326–331.
26. Maranho, L. T., Galvão, F., Preussler, K. H., Muniz, G. I. B., & Kuniyoshi, Y. S. (2006). *Acta Botanica Brasilica*, *20*, 615–624.
27. Udo, E. J., & Feyemi, A. A. A. (2001). *Journal of Environmental Quality*, *4*, 537–540.
28. Hou, F. S. L., Leung, D. W. M., Milke, M. W., & Macpherson, D. J. (1999). *Environmental Technology*, *20*, 413–418.
29. Siddiqui, S., & Adams, W. A. (2002). *Environmental Toxicology*, *17*, 49–62.
30. Salanitro, J., Dorn, P., Hueseman, M., Moore, K., Rhodes, I., Rice, L., et al. (1997). *Environmental Science & Technology*, *31*, 1769–1776.
31. Rivera-Cruz, M. C., & Trujillo-Nárcia, A. (2004). *Interciencia*, *29*, 369–376.
32. Teal, J. M., & Kanwisher, J. W. (1966). *Journal of Experimental Botany*, *17*, 355–361.

33. Luxmoore, R. J., Stolzy, L. H., & Letey, J. (1970). *Agronomy Journal*, *62*, 317–322.
34. Smith, W. H. (1990). In A. A. Lucier & S. H Haines (Eds.), *Mechanisms of forest response to acidic deposition* (pp. 188–241). New York: Springer.
35. Merkl, N., Schultze-Kraft, R., & Infante, C. (2005). *Water, Air, and Soil Pollution*, *165*, 195–209.
36. Merkl, N., Schultze-Kraft, R., & Infante, C. (2004). *Bioremediation Journal*, *8*, 177–184.
37. Schnoor, J. L., Licht, L. A., Mccutcheon, S. C., Wolfe, N. L., & Carreira, L. H. (1995). *Environmental Science & Technology*, *29*, 318–323.
38. Reilley, K. A., Banks, M. K., & Schwab, A. P. (1996). *Journal of Environmental Quality*, *25*, 212–219.
39. Liste, H. H., & Alexander, M. (2000). *Chemosphere*, *40*, 11–14.
40. Olson, P. E., Reardon, K. F., & Pilon-Smits, E. A. H. (2003). In S. C. McCutcheon & J. L. Schnoor (Eds.), *Phytoremediation: transformation and control of contaminants* (pp. 317–354). New York: Wiley.
41. Spriggs, T., Banks, M. K., & Schwab, P. (2005). *Journal of Environmental Quality*, *34*, 1755–1762.
42. Gudin, C., & Syratt, W. J. (1975). *Environmental Pollution*, *8*, 107–112.
43. Pivetz, B. E. (2001). *Phytoremediation of contaminated soil and ground water at hazardous waste sites*. EPA Ground water issue (EPA/540/S-01/500).
44. Trapp, S., & Karlson, U. (2001). *Journal of Soils and Sediments*, *1*, 1–7.
45. Singh, O. V., & Jain, R. K. (2003). *Applied Microbiology and Biotechnology*, *63*, 128–135.
46. Olson, P. E., & Fletcher, J. S. (2000). *Environmental Science and Pollution Research International*, *7*, 195–204.
47. Mackey, C. V., & DePuit, E. J. (1985). *Reclamation & Revegetation Research*, *4*, 1–16.
48. Rowell, M. J., & Florence, L. Z. (1993). *Soil Biology & Biochemistry*, *25*, 1499–1511.
49. Baker, J. M. (1979). In R. L. Jeffries & A. J. Davy (Eds.), *Ecological processes in coastal environments* (pp. 529–542). London: Blackwell Scientific.
50. Pilon-Smits, E. (2005). *Annual Review of Plant Biology*, *56*, 15–39.