Research Article

The potential association of *Echinochloa polystachya* (Kunth) Hitchc. with bacterial consortium for petroleum degradation in contaminated soil

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Abstract
In recent years, environmental impacts related to the contamination of ecosystems by petroleum have become frequent. In contact with the environment, petroleum can cause toxic effects in the biodiversity and on human health and compromise both water and land resources. Among the strategies to overcome this issue, bioremediation stands out as viable and promising alternative for environmental decontamination. To bioremediate petroleum-contaminated sites, phytoremediation and bioaugmentation techniques can be used. Thus, this research aimed to evaluate through a pot experiment four bioremediation strategies: (1) natural attenuation, (2) phytoremediation with *Echinochloa polystachya*, (3) bioaugmentation with bacterial consortium and (4) bioaugmentation-assisted phytoremediation, for the treatment of a co-contaminated soil presenting 100 g kg^{-1} of petroleum. In addition, two control treatments were carried out with substrates not contaminated with petroleum: (5) control with *E. polystachya* and (6) control treatment with bacterial consortium and *E. polystachya*. The experiment lasted 60 days in a greenhouse. The survival rate of *E. polystachya* was 100% in the contaminant tolerance aspect, resulting in increased stomatal density and aerenchyma, affecting few parameters of the plant, which demonstrate its phytoremediation capacity. In all treatments, petroleum degradation occurred. The highest degree of total petroleum hydrocarbon removal was obtained for contaminated soil cultivated with *E. polystachya* (phytoremediation), followed by contaminated soil cultivated with *E. polystachya* and bacterial (bioaugmentation-assisted phytoremediation treatment) and contaminated soil treated with bacterial consortium (bioaugmentation). Natural attenuation was less effective, proving the efficiency of the phytoremediation by *E. polystachya* and bacterial consortium, that responded positively to the stresses generated by contamination. However, further studies should direct to aim understanding the metabolic processes involved in the degradation and that these approaches to assist in environmental decontamination.

Keywords Petroleum hydrocarbons · Rhizosphere · Bioaccumulation · Phytotoxicity · Phytotoxicity · Contamination

1 Introduction

Petroleum is the world's leading energy matrix, and it requires extensive logistics for production, transportation, storage and marketing [36]. With the intense exploration of petroleum, its presence in the environment has become increasingly common, whether through accidental spills or incorrect disposal of the generated waste [52]. The arrival of this contaminant in the environment is one of the major causes of water and soil pollution, which directly affects the fauna and flora. In addition to being a threat to ecosystems, it can cause serious damage to human health

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due to its high carcinogenic and neurotoxic potential [33]. Therefore, to reconcile the increase in petroleum demand with environmental protection, it is necessary to understand which techniques can remediate contaminated environments.

Petroleum consists, predominantly, of aliphatic or aromatic hydrocarbons, in addition to resins and metals such as nickel, iron and vanadium [50] and may persist in the environment for several years due to the slow biodegradation of its components, such as aromatic molecules, which have a high molecular mass [5]. Thus, it is necessary to apply techniques capable of accelerating the degradation of this contaminant when present in the soil, such as the use of microorganisms and plants that together can bring better results [49]. This method includes natural attenuation, bioaugmentation and phytoremediation techniques, which are the most effective approaches and that present more cost–benefit ratio in the treatment of petroleum-contaminated soils [16].

In environments contaminated with petroleum hydrocarbons, the phytoremediation strategy employed is rhizo-degradation, an efficient technique for environmental decontamination, but that lacks a better understanding, especially about the interactions that occur between the plant, the microorganisms and the contaminant [22, 47]. In this technique, there is a synergy between the plant and the microorganisms that live in the rhizosphere, i.e., the bacteria that survive in symbiosis with the plant. This synergy promotes several ecological functions, such as the biological nitrogen fixation and other biogeochemical processes [26, 41]. Thus, they also provide increased nutrient absorption and tolerance to abiotic stress, and protection against pathogens.

Although efficient, the use of only one microorganism may have limited efficiency in bioremediation, since there may be competition with local microorganisms [16]. Therefore, the use of bacterial consortia, formed by strains isolated from different microorganisms previously selected due to their characteristics, can be more effective in removing contaminants, since there is a mutual and synergistic cooperation between the chosen bacteria [23, 37].

The plants, in turn, favor the action of microorganisms because they provide oxygen and nutrients for microbial growth, which enhances the degradation of petroleum hydrocarbons. However, plants may be directly or indirectly affected by petroleum in the soil [20], resulting in developmental [25, 28], structural [9, 10, 40] and physiological changes [45]. This occurs because an environment contaminated with petroleum exposes the plant to stresses, such as chemical, hydric (caused by the hydrofibricity of contaminants) and oxidative (excessive free radicals) ones, in addition to greater soil compaction and loss of nutrients [46].

Thus, the choice of plant is crucial for a successful phytoremediation technique, and to this end, it is necessary to understand the anatomical and physiological characteristics of the species. The plant chosen for phytoremediation must be resistant to the contaminant and have high biomass production capacity [11]. These two characteristics are present in *Echinochloa polystachya* (H.B.K.) Hitchcock (Poaceae), which has the capacity to phytoremediate petroleum-contaminated environments, mainly due to the microorganisms present in its rhizosphere [8, 35].

Therefore, by combining the phytoremediation plant with the bioaccumulation of microorganisms present in the rhizosphere, it is possible to increase the efficiency of the remediation technique on petroleum-contaminated soils. Thus, the objective of this study was to evaluate the effects of petroleum-contaminated soil inoculated with bacterial consortium on the growth, development and structural organization of *Echinochloa polystachya*, as well as to compare the degradation rate of petroleum hydrocarbons in the different treatments, in which the plant was cultivated with and without the bacterial consortium.

2 Materials and methods

2.1 Obtaining the bacterial consortium

To obtain the bacterial consortium, microspheres were produced from the bacterial biomass developed by Homan et al. [27], containing four species of bacteria extracted from the rhizosphere of *Echinochloa polystachya*: *Stenotrophomonas acidaminiphila*, *Bacillus subtilis*, *Bacillus pumilus* and *Microbacterium aerolatum*.

This solution was diluted in 1% alginate powder and then dripped with a 10 mL syringe arranged in a programmable pump, calibrated to produce drops every 0.5 mL min^{-1}. The drops were released in a 0.5% calcium chloride solution ($CaCl_2$) and then placed in a 1% acetic acid and 0.4% chitosan powder solution, where they remained for 40 min and were stirred every five minutes. Finally, the spheres were recovered using a stainless steel sieve (0.5 mm) and washed with distilled water.

The microspheres were then stored in plastic containers at −4 °C for 24 h. To increase the durability of the consortium, the product was dehydrated with the help of a freeze dryer until the microspheres were completely dried. Each microsphere weighed approximately 0.0076 G. Thus, 0.22 g of consortium was applied in each inoculated treatment, containing a total of 1.5×10^3 UFC.g^{-1} among the four different types of bacteria present in the bacterial consortium.

2.2 Establishment of the experiments

The study was carried out in a greenhouse at Positivo University (UP) with a structure covered with a transparent polyethylene film, activated against ultraviolet rays, with thickness of 150 micrometers, located in Curitiba, Paraná, south of Brazil (25°26′48.58″S, 49°21′31.87″W; 910 m a.s.l.). The experiments were conducted in temperature between 25 and 30 °C, relative humidity between 85 and 90% and 12 h of uninterrupted darkness and 12 h of sunlight every day. The collected soil underwent 0.5 mm mesh screening and was contaminated with 100 mg kg^{-1} of petroleum.

The petroleum used for establishing the experiment presented the following characteristics: relative density (20/4 °C) 0.90; density (°API) 23.8; total acid number 1.02 (mg KOH g^{-1}); salt content 202 (mg NaCl L^{-1}); water and sediment content 0.60%; saturated hydrocarbons 49.8%; aromatic hydrocarbon 25.4%; hydrocarbon resins 22.4%; and asphaltenes 2.4%. The plant treatments received a stake with two buds of *Echinochloa polystachya*.

Subsequently, these substrates were placed in polyvinyl chloride (PVC) vessels and six treatments were established (Fig. 1), with six repetitions each: (1) contaminated soil cultivated with *E. polystachya* and bacterial consortium (PPC); (2) contaminated soil cultivated with *E. polystachya* (PP); (3) contaminated soil treated with bacterial consortium (PC); (4) natural attenuation; (5) control treatment with *E. polystachya;* and (6) control treatment with bacterial consortium and *E. polystachya*. with six repetitions each.

Each treatment was repeated six times, being evaluated over a 60-day trial period. The plants were irrigated daily at field capacity of 85%. In this phase, aspects related to tolerance, growth and development of plants were analyzed. All samples were measured weekly with a metric tape for stem length. The plants were uprooted 60 days after the establishment of the experiments.

2.3 Development of *Echinochloa polystachya*

The determination of the chlorophyll content was based on the method proposed by Barnes et al. [6]. 28 mg obtained from fresh leaf samples collected from plants exposed to different treatments was preserved in tubes. To each sample, 5 ml of dimethyl sulfoxide was added. The tubes remained in water bath for 6 h, until the complete extraction of the pigment. The material absorbances were read using a spectrophotometer (Varian Cary 50B10) at 648 nm and 665 nm wavelengths. The respective absorbance values obtained were applied in Eqs. (1)–(3).
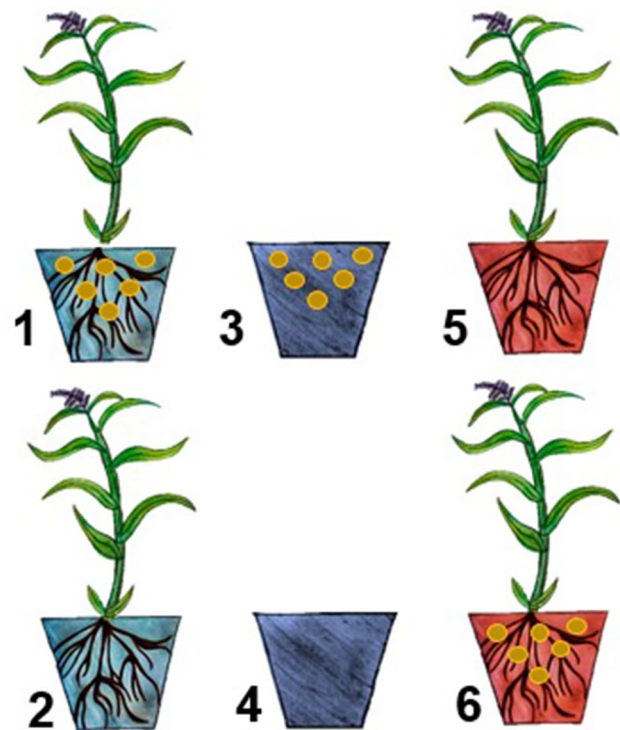
$$Ca = 14.85A648 - 5.14A648 \tag{1}$$

Fig. 1 Representative scheme of the treatments, (**1**) contaminated soil cultivated with *E. polystachya* and bacterial consortium (PPC); (**2**) contaminated soil cultivated with *E. polystachya* (PP); (**3**) contaminated soil treated with bacterial consortium (PC); (**4**) natural attenuation; (**5**) control treatment with *E. polystachya;* (**6**) control treatment with bacterial consortium and *E. polystachya*

$$Cb = 25.48A648 - 7.36A665 \tag{2}$$

$$C(a + b) = 7.49A665 + 20.34A648 \tag{3}$$

Ca = Chlorophyll a, Cb = Chlorophyll b, and C $(a + b)$ = Total chlorophyll. For unit conversion, the values calculated for Ca and Cb were multiplied by the volume of solvent that was used (5 mL) and divided by the leaf weight of each test tube (28 mg), obtaining the concentration of each pigment per unit of leaf weight in ml.mg^{-1}. The values obtained for chlorophyll a and b were multiplied by 1.121 and 1.104 (molecular weight), the unit being expressed in mmol.mg^{-1}.

2.4 Stomatal density

The stomatal density in individuals exposed to petroleum and control was determined according to the methodology described by Franklin [21], and the stomata were counted in an area of 1 mm^2 under a photonic microscope system (Olympus—CX41RF).

2.5 Anatomical analysis of *Echinochloa polystachya*

The analyses of the structural organization of the plants were performed on roots samples collected at 2 cm from the apex, and on leaves collected from the 4th stem node. These samples were fixed with FAA 70 for 48 h as described by Johansen [30] and kept in 70% ethanol until final processing [7]. The aerial and root biomass were determined after drying the material at 70 °C until constant weight.

The samples were embedded in glycol methacrylate (JB-4) [19] for the production of the permanent slides with synthetic resin (Entellan®). The sectioning was done using a rotating microtome, and the material was stained with toluidine blue. After this procedure, the slides were analyzed under an optical microscope, and the characteristics of the structural organization of *E. polystachya* leaves and roots in 0.7 μm cross sections were observed.

2.6 Degradation of petroleum hydrocarbons

To analyze petroleum degradation, 5 g of soil was collected from each treatment in the rhizosphere region. The plants were removed from the pots, and the soil near the roots was collected. In the treatments without plants, soil was collected in the same region. After that, 2 g of anhydrous sodium sulfate (Na_2SO_4) was added for the complete absorption of water from the samples, as well as 5 mL of dichloromethane solvent (CH_2Cl_2) according to the chromatography standard. The samples were placed on an automatic shaker for 5 h at 300 rpm, and then, the material was centrifuged for 15 min at 3000 rpm.

After these procedures, all samples supernatant was removed with Pasteur pipettes. Subsequently, 5 mL of dichloromethane was added to the samples again and the liquid part was removed until the complete extraction of the petroleum. The samples were stored separately in amber glass and remained under refrigeration. Afterward, gas chromatography analysis was carried out separately with 0.5 mL of each sample to compare petroleum degradation in the different treatments. The chromatograph used had an HP-5 capillary column (5% phenyl)—methylpolysiloxane (30 m, 25 mm id, 0.25 μm thick). Each compound was identified based on its retention time, and the area reduction percentage was quantified. Natural attenuation peak areas (NA) were used as a point of reference for the remaining compounds (100%) in the treated systems.

2.7 Statistical analyses

All data were analyzed in the BioStat 5.3 software. Mean and standard deviations were calculated. With these results, data distribution analysis was performed using the Shapiro–Wilk test. Subsequently, the one-way ANOVA test

and the differences between means were compared using the Tukey test, at 5% significance ($p \leq 0.05$).

3 Results

3.1 Development of *Echinochloa polystachya*

The survival rate reached 100% in all treatments, demonstrating the tolerance of *E. polystachya* to a petroleum-contaminated soil. The results regarding growth, aerial biomass, root biomass, chlorophyll a, chlorophyll b, total chlorophyll, stomatal density and stomatal characteristics are shown in Table 1.

Higher growth and biomass of the aerial part and root were observed in plants cultivated in petroleum-contaminated soil in a bacterial consortium in relation to the control treatment. On the other hand, the results obtained for the chlorophyll content demonstrate that there was no significant reduction in the content of photosynthesizing pigments in plants exposed to contamination (Table 1).

3.2 Stomatal density

Echinochloa polystachya leaves are amphistomatous. When viewed from the front, the epidermal cells have slightly sinuous rectangular-shaped walls on the adaxial and abaxial surfaces and are of various sizes. The stomata have dumbbell-shaped guard cells arranged in parallel rows (Fig. 2) and are located at the same level as the other epidermal cells. The stomatal density is 78 stomata per mm^2 in control individuals (Table 1). In relation to the individuals exposed to petroleum, there was an increase of up to 24% in stomatal density (Fig. 2a) compared to control individuals (Fig. 2b). However, no significant differences were found in the size of the stomata in the different treatments, showing that petroleum does not affect the development of the plant in this parameter.

3.3 Anatomical description of *Echinochloa polystachya*

In cross section, the *E. polystachya* epidermis is uniseriate, formed by rounded cells with thick walls arranged at different heights, being visible through smaller cells above the fiber bundles. Throughout the mesophyll, epidermis and adaxial surface, it is possible to observe the presence of bulliform cells, which are large epidermal cells that usually occur in groups. The main vein, in cross section, is straight on the adaxial surface and has a large protuberance on the abaxial surface. In the straight region, the subepidermal cells constitute the pulvinar bands, whose cells have thin walls and are larger than in other regions

Table 1 Growth, biomass, chlorophyll content, stomatal density, ostiole and guard cells size of the *Echinochloa polystachya* stomata cultivated in the different treatments

Parameters	CT—plant	CT—plant and consortium	Plant on soil contaminated with petroleum	Plant on soil contaminated with petroleum and consortium
(cm)				
Stem length	22.5 ± 12.33[acd]	34.27 ± 18.84[bcd]	26.13 ± 5.49[cbd]	43.14 ± 15.52[dbc]
(g)				
Aerial biomass	3.19 ± 1.44[ad]	2.69 ± 15.74[bcd]	1.72 ± 0.58[cb]	4.07 ± 1.66[dab]
Root biomass	0.71 ± 0.41	2.11 ± 0.90	1.40 ± 0.40[cb]	4.06 ± 0.99[ab]
mmol/mg				
Total chlorophyll	20.25 ± 6.05	18.25 ± 3.59	19.05 ± 2.11	18.37 ± 4.09
Chlorophyll a	17.64 ± 5.24	16.00 ± 4.11	16.83 ± 1.68	16.24 ± 8.82
Chlorophyll b	2.60 ± 1.75	2.25 ± 1.72	2.22 ± 0.66	2.13 ± 1.29
uni./mm^2				
Stomatal density	78.00 ± 15.74[ab]	84.00 ± 8.76[bacd]	97.00 ± 21.52[cbd]	96.00 ± 8.39[dbc]
μm				
Ostiolum length	31.16 ± 2.75[ac]	38.5 ± 3.34[bac]	32.66 ± 3.65[cbd]	34.83 ± 1.46[da]
Guard cell	23 ± 5.65[abcd]	20.57 ± 2.34	20.78 ± 1.62[ca]	22.35 ± 2.13

*Different letters next to the values in the same line indicate significant statistical differences by the Tukey test ($p \leq 0.05$)
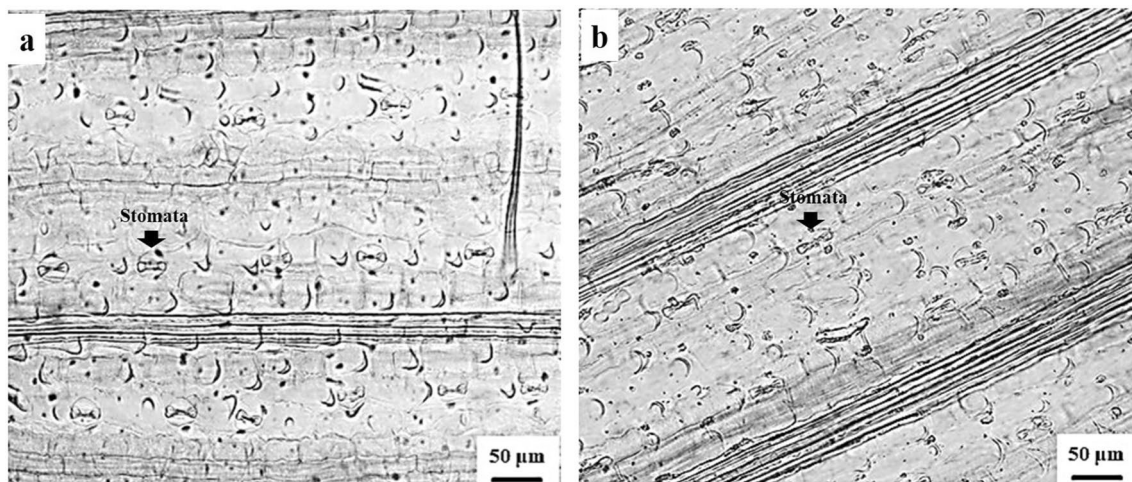

Fig. 2 Epidermis of *E. polystachya* with stomata arranged in parallel rows; **a** increased stomatal density in individuals exposed to petroleum contamination; **b** control treatment with *E. polystachya*

of the leaf, exerting motive function. In the region of the protuberance, the epidermal cells present thickened and lignified walls. In the median region of the limbus, 7 to 8 layers of cells and a large volume of aerenchyma are found (Fig. 3a).

In the mesophyll, there is a fundamental parenchyma and in the abaxial surface there are scattered fiber bundles in the chlorenchyma. The vascular bundle, which is normally oriented, does not differ from the others. Epidermal cells are larger and present thickening of the outer periclinal wall. The vascular bundles are collateral and closed (Fig. 4), and there are two types: smaller and

larger ones, alternately arranged along the mesophyll. The largest bundles are surrounded by a double sheath, the outer one being the parenchyma and the inner one the sclerenchyma. The vascular bundles are formed usually by two large metaxylem vessels and protoxylem.

The individuals exposed to petroleum did not show changes in the number of central nerve cells layers. However, it is possible to see that control individuals have larger cells (Fig. 3a, b), while plants grown in petroleum-contaminated soil (Fig. 3c, d) had smaller and more compact cells.
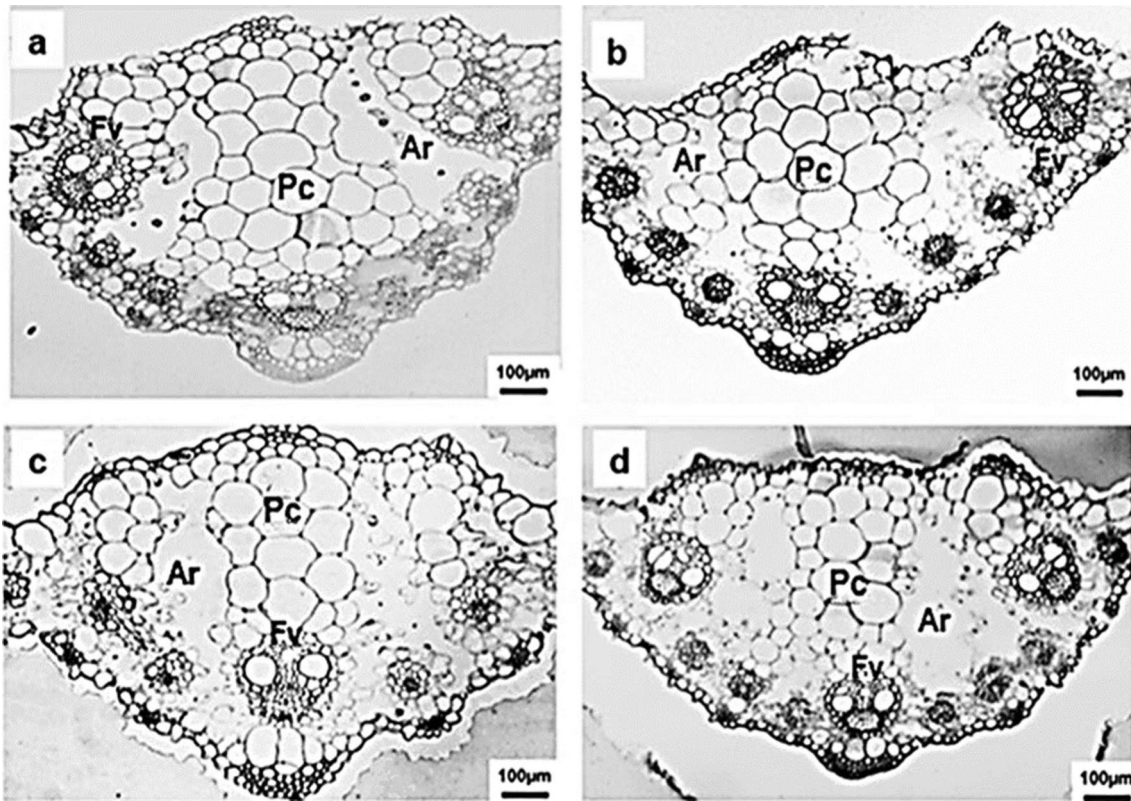
Fig. 3 Cross section of the main vein of *E. polystachya*. **a** Control treatment with *E. polystachya*; **b** control treatment with bacterial consortium and *E. polystachya*; **c** treatment contaminated soil cultivated with *E. polystachya*; **d** treatment contaminated soil cultivated with *E. polystachya* and bacterial consortium. Pc, chlorophyll parenchyma; Air, aerenchyma between parenchymal cells; Fv, vascular bundles
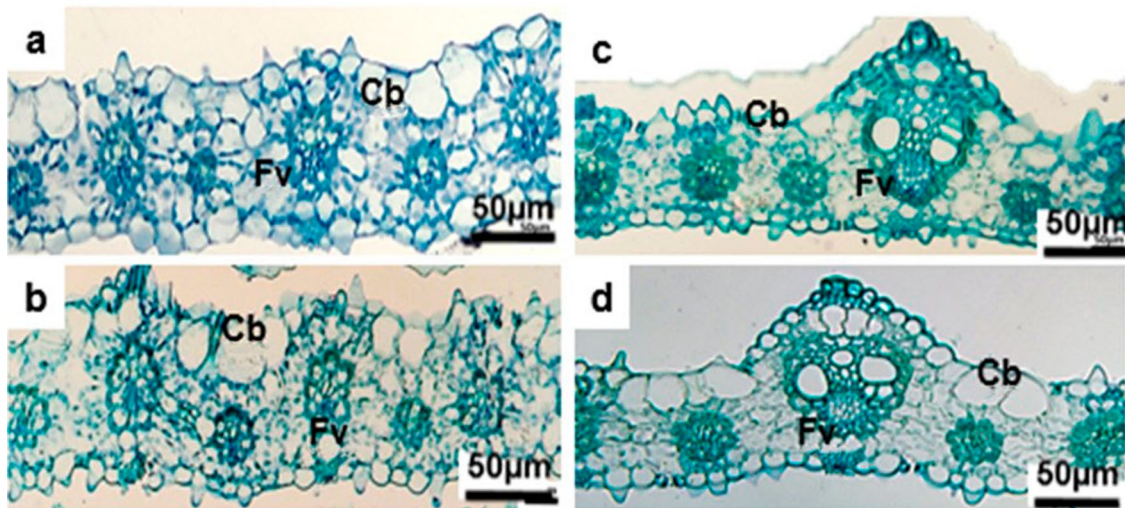

Fig. 4 Cross section of the *E. polystachya* mesophyll. **a** Control treatment with *E. polystachya*; **b** control treatment with bacterial consortium and *E. polystachya*; **c** treatment contaminated soil culti- vated with *E. polystachya*; **d** treatment contaminated soil cultivated with *E. polystachya* and bacterial consortium. Cb, bulliform cells; Fv, vascular bundle

The roots of *E. polystachya* have a layer of outer cells, the exodermis, in which the cell walls are more lignified and thicker. In all roots, few trichomes were observed. The root cortex, located under the exodermis, consists of 9 to 10 layers of parenchymal cells, which were rounded and presented a solid disposition. In the cortex, we also identified the presence of aerenchyma, next to cells with collapsed walls. The innermost portion of the cortex is in contact with the uniseriate endodermis, which is a thick U-shaped layer and delimits the central cylinder. The pericycle is located under the endoderm and consists of a uniseriate layer of cells (Fig. 5). This cylinder is responsible for the growth of lateral roots and for surrounding the vascular bundles of the root. The vascular system consists of the poles of the protoxylem to the inside of the metaxylem, where the phloem is found near the outermost ring.

In individuals exposed to petroleum, a small increase in the number of cells in the cortex was found, with 10 to 12 layers of cells. We have also observed a higher amount of aerenchyma in these individuals.

3.4 Degradation of petroleum hydrocarbons

The efficiencies of all treatments can be seen in Table 2, where the efficiency of the degradation of hydrocarbons of petroleum among the different treatments applied: contaminated soil cultivated with *E. polystachya* (PP); contaminated soil treated with bacterial consortium (PC); and contaminated soil cultivated with *E. polystachya* and bacterial consortium (PPC), in relation to the petroleum degradation obtained in natural attenuation (AT).

The natural attenuation treatment had the lowest degradation rate in relation to other treatments, with no significant difference in degradation in some retention times. The treatment only with bacterial consortium (PC) had less degradation efficiencies of petroleum hydrocarbons when compared to the treatments in which plants were used. However, in some petroleum chains, the treatment with PC showed a degradation of up to 57% compared to natural attenuation.

The treatments in which *E. polystachya* was used had high rates of degradation. Only the phytoremediation technique showed degradation in almost all retention
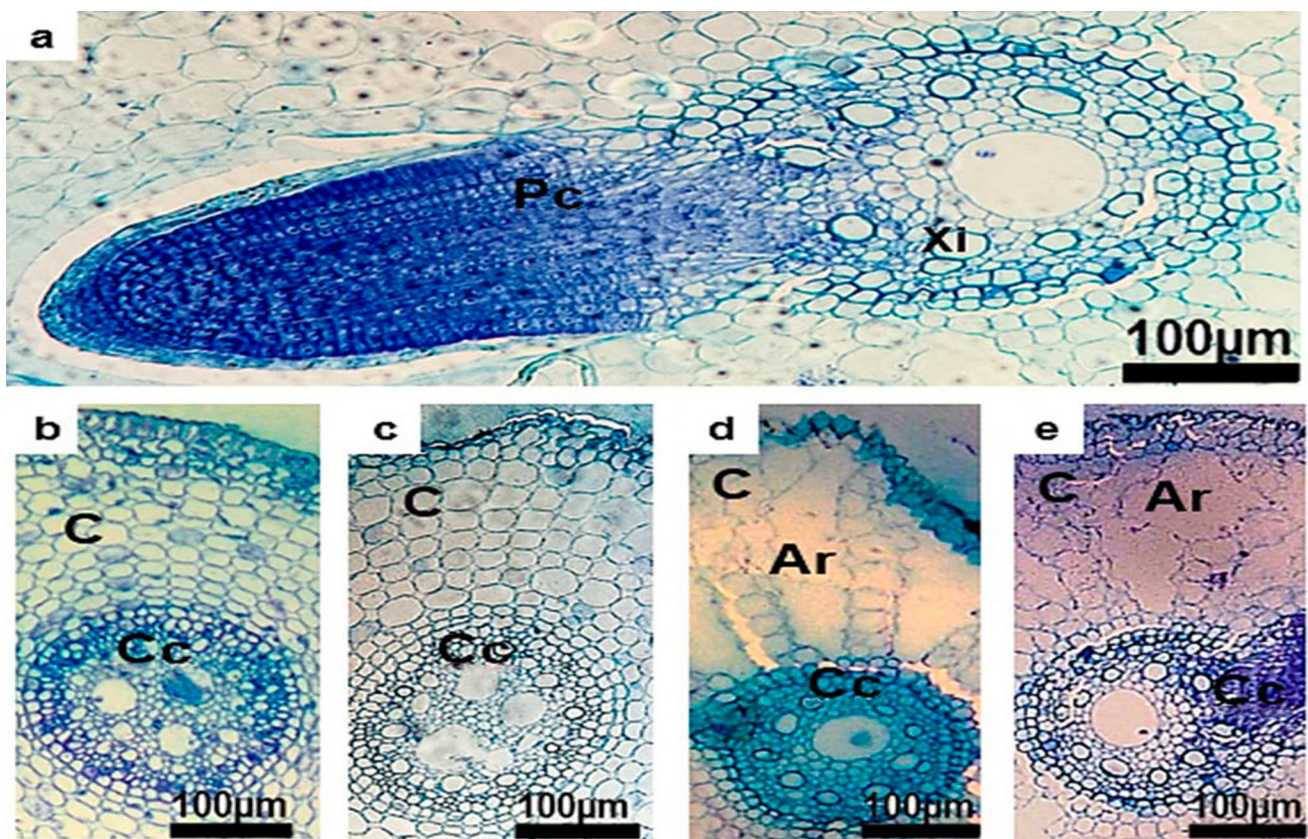

Fig. 5 Adventitious root of *E. polystachya*. **a** Activity of the pericycle and of the endoderm with U-shaped reinforcement; **b** control treatment with *E. polystachya*; **c** control treatment with bacterial consortium and *E. polystachya*; **d** treatment contaminated soil cultivated with *E. polystachya*; **e** treatment contaminated soil cultivated with *E. polystachya* and bacterial consortium. Pc, pericycle; Xi, xylem; C, cortex; Cc, central cylinder; Ar, aerenchyma

Table 2 Efficiency in petroleum degradation among the different treatments, compared by retention time and area size

Retention time (min)	AT	PP	%	PC	%	PPC	%
3.422	6782[a]	3475[bcd]	49	5684[cbd]	0	8619[dbc]	0
5.003	8935[ab]	5281[bacd]	41	6798[cdb]	0	7639[dbc]	15
5.876	12497[ab]	5475[bacd]	56	12512[cbd]	0	10314[dbc]	17
6.525	18431[a]	8408[bcd]	54	13642[cbd]	0	12678[dbc]	31
7.089	28131[a]	12499[bcd]	56	12980[cbd]	52	19339[dbc]	31
7.512	23622[a]	11347[bcd]	52	21069[cbd]	0	17711[dbc]	25
8.572	46078[abcd]	30447[bacd]	34	29716[cabd]	0	47796[dabc]	0
9.510	64431[abd]	34767[bacd]	41	73855[cbd]	26	30388[dabc]	48
10.110	8547[ac]	26383[bc]	69	24671[cabd]	0	47620[dc]	44
10.546	123327[acd]	47614[bcd]	51	15682[cabd]	0	24625[dabc]	54
12.027	21043[ab]	15572[bacd]	35	12811[cbd]	21	18748[dbc]	39
12.578	21406[abd]	9029[bacd]	65	23372[cbd]	0	17010[dabc]	40
13.542	24568[a]	22464,5[bcd]	9	16756[cbd]	0	6151[dbc]	75
16.085	13243[a]	13415[bcd]	0	9306[cbd]	0	8221[dbc]	38
17.236	65573[ad]	20929[bcd]	68	33489[cbd]	0	74231[dabc]	0
18.086	33507[ab]	25596[bacd]	24	52563[cbd]	57	39091[dbc]	0
19.339	37624[abcd]	19053[bacd]	51	17285[cabd]	0	51279[dabc]	0
20.265	31658[abd]	14830[bacd]	53	13364[cbd]	0	46338[dabc]	0
22.717	13248[abd]	5872[bacd]	56	10558[cbd]	0	22300[dabc]	0
24.367	5532[abc]	3000[bacd]	46	2936[cabd]	0	7078[dbc]	0

Natural attenuation (AT) was compared to the remaining treatments: *PP* contaminated soil cultivated with *E. polystachya*; *PC* contaminated soil treated with bacterial consortium; and *PPC* contaminated soil cultivated with *E. polystachya* and bacterial consortium

% Efficiency in petroleum degradation

*Different letters next to the values in the same line indicate significant statistical differences by the Tukey test ($p \le 0.05$)

times, with an efficiency of up to 69% when compared to natural attenuation. However, the treatment in which bioaugmentation and phytoremediation (PPC) techniques were used together obtained a degradation efficiency of up to 75%.

4 Discussion

4.1 Plant analyses

The results showed that the presence of petroleum in the soil promotes an increase in the growth, mainly stem length and root biomass of *E. polystachya*. This one can be observed especially in treatment "plant on soil contaminated with petroleum and consortium" where the bacterial consortium further increased the growth and biomass increment (Table 1). When present in environments contaminated with oil, some plants can develop responses to the scarcity of water and oxygen they are being subjected to, as changes in the roots, stems and leaves. These changes can be interpreted as strategies developed by plants to tolerate the toxic effects of petroleum. Lopes and Piedade [35] and Biazão et al. [8] found similar results when they evaluated the growth of *E. polystachya* cultivated in substrates contaminated with petroleum.

The inoculation of the bacterial consortium promoted the increase in *E. polystachya* biomass. Plants provide oxygen and release through their roots, substances containing sugars, amino acids and organic acids. These, as exchange, are metabolized by microorganisms, thus improving the degradation of the pollutant [47]. Moreover, the organic compounds in the pollutant can be used as a carbon source in this process, which is responsible for reducing the effects of the contaminants retained in the soil [32]. Thus, the microorganisms stimulate the growth and development of plants to improve the uptake of water and nutrients from the soil [24, 31].

Maqbool et al. [39], in a study on the effect of the inoculation by free and immobilized bacterial culture on the rhizodegradation of petroleum-polluted soil using *Sesbania cannabina* plant, found root biomass increased with the plant growth, during experimental period between

the treatments with different petroleum-polluted soil concentrations.

The chlorophyll content results showed that there is no significant difference in the pigment content of plants grown in petroleum-contaminated soil compared to control individuals. Normally, the soil contaminated by petroleum decreases the chlorophyll content and can lead to a reduction in the concentration of photosynthetic pigments in the plant [45], because hydrocarbons tend to accumulate in the chloroplasts [4]. The level of chlorophyll reduction in plants can be used as an indicator of petroleum contamination [2, 42]. However, our results demonstrate that petroleum does not affect the development of *E. polystachya* in this respect.

The increase in stomatal density shows that the plants developed mechanisms to survive the environmental conditions generated by the petroleum present in the soil (Table 1). Maranho et al. [40] repost that the toxic effects of petroleum can affect the morphology of the leaves with an increased number of intercellular spaces and increased stomata density. According to Larcher [34], this increase corresponds to an adaptive change under conditions of water scarcity, in which the stomata may be smaller in size and higher in density. This modification enables a faster reduction of perspiration, facilitating the closure of the stomata. However, in the present study, no significant differences were found for *E. polystachya* regarding the size of the stomata (Table 1) in the different treatments, not being affected by petroleum in this parameter. Zhang et al. [53], studying *Zea mays* L. (Poaceae) in soil contaminated by petroleum, found that stomatal density was negatively correlated with the petroleum level in soil.

In the anatomical analyses, no differences were found in the number of cells in the leaf midrib, but it was possible to visualize a decrease in the size of the parenchyma cells in relation to the control individuals (Fig. 4). The higher volume of intercellular spaces forming the aerenchyma (Fig. 3) is a response mechanism for plants grown in contaminated soil, which store larger amounts of oxygen inside and decrease the number of surrounding cells to survive the environment with petroleum. The same result was found when the root anatomy was analyzed, which evidenced a greater aerenchyma volume (Fig. 5), consistent with the results found for the higher root biomass in individuals exposed to petroleum (Table 1). The anoxic environment formed by petroleum also induced an increase in intercellular spaces, a reduction in the thickness of the central cylinder and an increase in the thickness of the root cortex (Fig. 5). Plants with dense and branched roots are considered suitable for the phytoremediation of organic pollutants as they provide a larger rhizospheric area, and thus a greater colonization of microorganisms [24, 51], since they provide oxygen to the rhizoplane.

Although aromatic hydrocarbons are degraded, the proportion of this process decreases in proportion to the low availability of oxygen in the contaminated environment. Phytoremediation technology uses the morphophysiological potential of plants to favor the degradation of pollutants. Plants participate in the remediation process through their roots, with the diffusion of oxygen into the sediment, where the microorganisms are present.

4.2 Degradation of petroleum hydrocarbons

Studies show that natural attenuation minimizes the concentration of the contaminant in the soil, but this technique involves only natural processes and may take years to partially or totally remedy a contaminated environment [29]. Therefore, it is important to intervene in this process in order to increase the degradation of petroleum in a given environment. The plant treatments showed better results when compared with the treatment with only bacterial consortium (Fig. 6). This is because the bacteria used depend on the rhizosphere of *E. polystachya* to develop, since they provide oxygen and other nutrients to the soil.

It was also possible to verify that the *E. polystachya* plant can phytoremediate independently from the bacterial consortium. In a revegetation study in petroleum-contaminated areas, Panchenko et al. [43] found that members of the Poaceae family were the most resistant to contamination, particularly *Echinochloa crus-galli*, which was found in areas with up to 200 g kg^{-1} of petroleum with high survival rates.

Elumalai et al. [17] cite that the bioaugmentation is a very promising technology for the removal of long-chain hydrocarbons and n-alkanes from oil-contaminated environments but requires highly efficient microbes.

When using the phytoremediation and bioaugmentation techniques together, it is possible to increase the efficiency of degradation, which has been verified by Escalante-Espinosa et al. [18] when using *Cyperus laxus* (order Poales) in soil contaminated with petroleum and inoculated with bacterial consortium, whose degradation was more efficient when compared to other treatments. According to Tahir et al. [48], microorganisms are essential for environmental recovery and restoration because they easily adapt to the stresses caused by the presence of contaminants in the environment and have the resistance capacity and metabolic potential inherited by natural selection.

Thus, according to Santos and Maranho [47], the application of bacterial consortia in petroleum-contaminated environments has brought satisfactory results. Additionally, according to Chandra et al. [15], the consortia allow associations between different species, increase in the enzymes capable of degrading petroleum hydrocarbons.
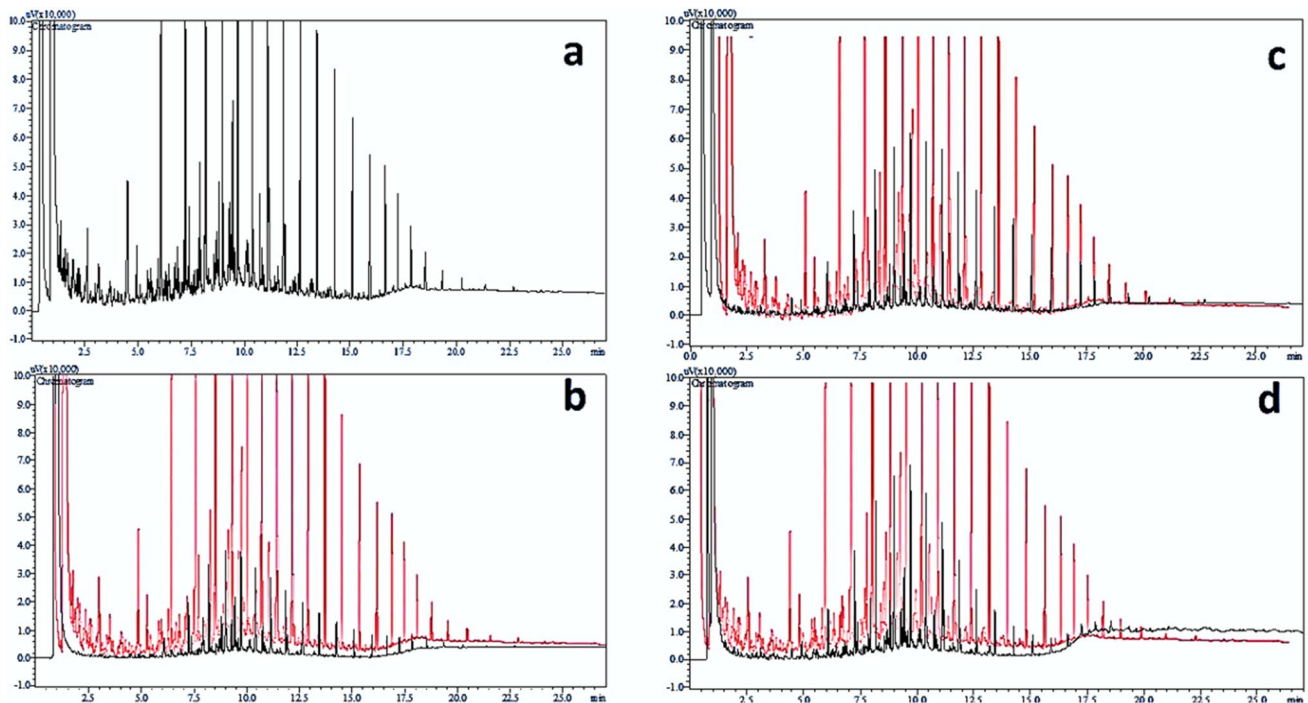
Fig. 6 Chromatograms of petroleum degradation, **a** natural attenuation; **b** contaminated soil cultivated with *E. polystachya* (PP); **c** contaminated soil treated with bacterial consortium (PC); **d** contaminated soil cultivated with *E. polystachya* and bacterial consortium (PPC). ****b**–**d** Natural attenuation (AT) in red was compared to the remaining treatments in black

Cerqueira et al. [14] showed that the microbial consortium composed by five bacteria (*Stenotrophomonas acidaminiphila*, *Bacillus megaterium*, *Bacillus cibi*, *Pseudomonas aeruginosa* and *Bacillus cereus*) degraded, in liquid medium, high concentration of petroleum compounds producing biosurfactant efficiently. The consortium used in this research was composed of *Stenotrophomonas acidaminiphila*, *Bacillus subtilis*, *Bacillus pumilus* and *Microbacterium aerolatum*.

Mangwani et al. [38] when investigating the biofilm formation and polycyclic aromatic hydrocarbons (PAHs) degradation found that the marine bacterium *Stenotrophomonas acidaminiphila* was able to degrade PAHs. The authors highlight its bioremediation process application. Parthipan et al. [44] cite that *B. subtilis* produces high amounts of biosurfactant and degradative enzymes such as alkane hydroxylase and alcohol dehydrogenase in the presence of crude oil as a substrate. Calvo et al. [13] evaluated the surfactant activity of a *Bacillus pumilus* strain isolated from oil sludge and found that this bacterium grew well in the presence of crude oil under aerobic conditions and utilized the oil compounds as carbon and energy source. Ali et al. [3] monitored the dynamics of the hydrocarbonoclastic bacterial communities in desert soil sample was saturated with crude oil, and they identified the bacterial species and found *Microbacterium aerolatum* as main contributor to the oil consumption.

While studying natural attenuation, phytoremediation and bioaugmentation using *Festuca arundinacea* and microbial consortium [12] found that the phytoremediation and bioaugmentation are effective in remediating saline soil contaminated by heavy crude oil. Agnello et al. [1] when evaluating the natural attenuation, phytoremediation, bioaugmentation and phytoremediation + bioaugmentation found that the highest degree of removal of total petroleum hydrocarbons from PHCs was obtained by combining plant–bacterial interaction, being the most advantageous option for oil treatment contaminated soil.

The interaction between plant and bacteria is the most advantageous option for the treatment of petroleum-contaminated soils. In recent years, scientists have been investigating how the various bacteria contribute to phytoremediation (rhizodegradation), and the effectiveness of these approaches has been mainly demonstrated under laboratory conditions (ex situ). Nevertheless, further studies are required considering practical applications (in situ) of bioremediation to contain environmental contaminations. This should enable a deeper understanding of the interactions between plant, bacteria, contaminant and

soil, and how to tailor each parameter to improve biodegradation efficiency and minimize its negative impacts. Therefore, increasing efforts should be conducted to select, identify and understand the role of rhizospheric microorganisms to expand the database regarding their petroleum-degrading potential to degrade petroleum and for bioprospection.

5 Conclusion

The use of *E. polystachya* for phytoremediation of soil contaminated with petroleum was proved effective. The plant responded positively to the stresses generated by contamination. All individuals survived contamination with little effect on their development and growth, showing the high resistance of this plant to contaminants. Rhizodegradation is a low-cost, easy to apply and effective technique in petroleum remediation. In the rhizosphere, there is a synergy between microorganisms and plants, creating a favorable environment for their growth.

Thus, when the phytoremediation and bioaugmentation techniques are used jointly, it is possible to obtain high rates of petroleum degradation. Microorganisms stimulate the bioavailability of the soil because they increase degradation rate and promote the partial or complete elimination of this contaminant in soils.

Compliance with ethical standards

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