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Salix rubens and *Salix triandra* Species as Phytoremediators of Soil Contaminated with Petroleum-Derived Hydrocarbons

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Abstract The petroleum industry activities provide potential risks to the environment because they can contaminate ecosystems with different organic compounds in the production chain. Several accidents with transport and handling of petroleum and related products occurred in urban areas with harmful effects to the quality of life and economy. In the 1990s, bioremediation and phytoremediation technologies as economically feasible alternatives to repair the environmental damage were developed. In this study, the potential of the willows *Salix rubens* and *Salix triandra* were evaluated with regard to the phytoremediation of soils contaminated with petroleum-derived hydrocarbons (total hydrocarbons and polycyclic aromatic hydrocarbons (PAHs)). The PAHs were quantified by extraction from soils and plants using dichloromethane under ultrasonication. The HPLC analysis was performed with GC/MSD equipment. The total hydrocarbons present in uncontaminated soil were quantified by the sum of animal/vegetable oils and greases and mineral oils and greases according to Standard Methods 5520 (1997). The two willows species *S. rubens* and *S. triandra* were resistant during the project development. In the contaminated soil, in which both species were planted, the total hydrocarbons concentration was reduced near 98 %. The PAHs content was remarkably reduced as well. Pyrene showed an initial concentration of 23.06 μg kg^{-1}, decreasing in most cases to 0.1 μg kg^{-1} or to undetectable levels. Chrysene decreased from 126.27 μg kg^{-1} to undetectable levels. Benzo[*k*]fluoranthene and benzo[*a*]pyrene concentrations had also showed a decrease from 28.44 and 3.82 μg kg^{-1}, respectively, to undetectable levels.

Keywords Contaminated soil · PAHs · *Salix* species · Phytoremediation · Phytodegradation

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1 Introduction

During the twentieth century, due to an expansion of the petrochemical industry, there was an increase in the production of chemicals, including fuels and solvents widely used. Over the years, oil has become an essential source of energy. The oil industry can cause damage to the environment because it can contaminate ecosystems with several organic compounds along the production chain. Oil is a fossil fuel of great significance to the world economy, but also represents an important environmental problem, because of its frequent introduction into the environment, not only in the form of fuel but also through the wide industrial

use of its derivatives (McCall and Pennings 2012; Jernelöv 2010). Therefore, soil contamination by oil and its derivatives has received extensive research in recent years. Oil and its derivatives are major environmental pollutants, along with heavy metals, organochlorines, and highly volatile compounds (Tonini et al. 2010; Ogbo and Okhuoya 2008; Patin 1982). An area contaminated with these pollutants can cause problems, including risks to human health, impaired quality of water resources, restrictions on land use, and serious damage to biota. There have been several oil spills around the world (Anastas et al. 2010; Aps et al. 2009; Davidson et al. 2008). Accidents often occur close to large population centers and impair the quality of life of the local inhabitants, as well as the economy (Oliveira 2004). Due to the growing ecological awareness and pressure for adequate environmental management of natural resources, private companies have taken control processes, such as environmental auditing and environmental management systems (Deotti 2005).

In the 1990s, viable technologies of bioremediation and phytoremediation became available as alternatives for environmental repair. Phytoremediation is a process that uses plant for biological treatment of both contaminated soil and water. Operating costs are very low, ranging from \$0.02 to 1.00 per m^3 of soil. The method may be applied in situ and used the sun as an energy source. This technology has been used to treat soils contaminated with metals (Pb, Zn, Cu, Ni, Hg, and Se), inorganic compounds (NO_3^-, NH^{4+}, and PO_4^{3-}), radioactive chemical elements (U, Cs, and Sr), and petroleum-derived hydrocarbons (benzene, toluene, ethylbenzene, and xylene and polycyclic aromatic hydrocarbons (PAHs)), among others (Silva et al. 2009; Bamforth and Singleton 2005; Anselmo and Jones 2005; Newman and Reynolds 2004; Cunningham et al. 1996). Phytoremediation is based on the use of plant species to extract, retain, immobilize, or degrade contaminants in water and soil (Diab 2008; McCutcheon and Schnoor 2003). Application of this technology in tropical countries is significantly enhanced due to the microorganism and plant diversity. The phytoremediation techniques provide good recovery of soils contaminated with heavy metals, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents, and toxic by-products of diversified industrial activities (Pires et al. 2003). PAHs are a family of compounds characterized by the presence of two or more condensed aromatic rings in their structure. These substances, as well as their nitrogenated and oxygenated derivatives, are widely distributed and found as components of complex mixtures in all types of environments (Netto et al. 2000). These compounds present a high health risk due to their chemical stability and high toxicity and because they are potentially carcinogenic (Andreoni et al. 2004; Cole 1994).

PAHs and their derivatives, normally, are associated with an increased incidence of various cancers in humans. Several components of this group, in their original form or decomposed, are able to directly react with DNA, making them potentially carcinogenic and efficient mutagenic agents. Human exposure to these compounds occurs mainly through environmental contamination (Netto et al. 2000). The US Environmental Protection Agency (1995) has established a list of 16 PAHs for prioritized environmental monitoring based on their carcinogenicity and occurrence.

The most stable PAHs, including pyrene, fluoranthene, benzo[*k*]fluoranthene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*cd*]pyrene, are known in the literature as tracers of vehicular traffic activity, except benzo[*a*]pyrene that is the most unstable substance. Fluorene, anthracene, phenanthrene, and pyrene are considered tracers of diesel engines, while fluoranthene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*cd*]pyrene are considered tracers of gasoline engine emissions (Ferraz 2005).

Another parameter frequently used to evaluate contamination from oil spills is the total petroleum hydrocarbon (TPH) concentration. This parameter provides information on the concentration of total hydrocarbons present in a given sample. The proposed definition of TPH, according to EPA, considers two concepts. The first is the fractionation of the oil mixture into separate constituents; thus, everything that is detected can be summed as the TPH. The other concept suggests that the TPH includes all hydrocarbons extracted from a sample and detected by a particular technique.

In this study, the potential of the willows *Salix rubens* and *Salix triandra* was evaluated for the phytoremediation of soils contaminated with petroleum-derived hydrocarbons (total hydrocarbons and PAHs).

2 Material and Methods

The study was developed in Canoas City, Rio Grande do Sul State, Brazil, and it was carried out in 36 months. It was a pilot experiment performed in

wooden boxes with 1.5×1.5×1.0 m (length, width, and height). The statistical model adopted was a 2^2 factorial in triplicate with completely randomized blocks, summing 12 experiments (boxes numbered from 1 to 12). The studied variables were a contaminated soil with hydrocarbons and PAHs, an uncontaminated soil (sandy clay soil taken from an area near Canoas City), and two willows species *S. triandra* and *S. rubens*. The species *S. triandra* was chosen because similar studies were performed by the Laboratory of Analytical Chemistry and Applied Ecochemistry, Ghent University, Belgium, where one of the authors visited in the year 2007 and saw the phytoremediation research with species of willow made by the researchers. Same studies developed by them demonstrated the effectiveness of the willow species in phytoremediation (Meers et al. 2007; Mertens et al. 2006; Vandecasteele et al. 2005a,b,c; Meers et al. 2005; Vervaeke et al. 2002). Although this species is a European variety, the authors found a farm in Santa Catarina State, Southern Brazil, which produces this species experimentally. This farm also produces the species *S. rubens*. It is a hybrid plant resulting from crossing two European species (*Salix alba* and *Salix fragilis*), which was introduced in Brazil, and they are more than half a century, spreading through the states of São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul (Moura 2002). Furthermore, Casa et al. (2007) indicate that the two species are resistant to attack by pests and leaf diseases, important features in the development of the experiment.

Figure 1 represents the boxes arrangement indicating the species planted and soil type. Each box was filled with approximately 4,000 kg of material. Both willows species were planted in two different materials. One of them (called UC soil) containing only uncontaminated soil. The other one (called C soil) containing a mixture of 80 % by weight with UC soil and 20 % by weight with contaminant. This contaminant is a sandy material with high dopant concentration of petroleum-derived hydrocarbons, one sample from an area accidentally impacted with this product. The six boxes containing UC soil were considered as a control. For soil pH correction, 16 kg of calcium carbonate and 5 kg of magnesium carbonate were added to the boxes. The mixture was prepared in a cement mixer and subsequently transferred to the boxes. Boxes with UC soil did not receive the correctives.

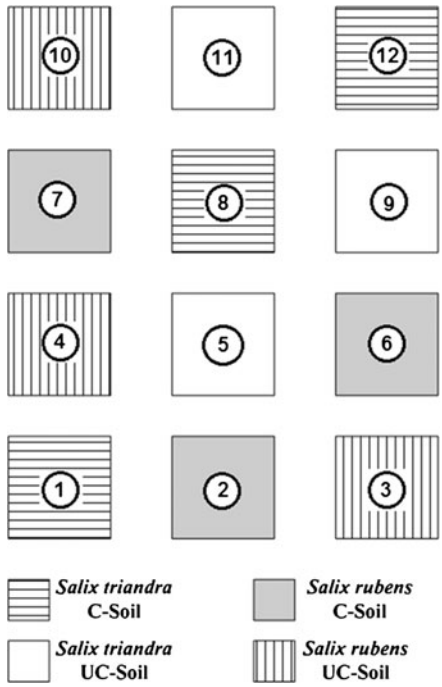

Fig. 1 Arrangement of the boxes indicating the species planted and soil type

2.1 Initial and Final Soil Sampling

Soil samples from 12 boxes were collected and analyzed before planting and 36 months after planting. The initial quantity collected was 2 kg. After homogenization, there was a reduction in each sample for analysis.

2.2 Sampling of the Willows Species

Two sampling of willows species occurred 18 and 36 months after planting. During the sampling, leaves, stems, and roots were collected. The leaves were collected from every plant in each box. For the stems and roots, three plants in each box were chosen randomly.

2.3 Quantification of PAHs in the C Soil and Willow Species

Plant and C soil samples were kept frozen until analysis. The analysis was performed by extracting the organic compounds from both the C soil and plants using dichloromethane and ultrasonic for 1 h, which was divided in three interspersed cycles of 20 min, with 5 min at rest. A volume of 5.0 mL of dichloromethane

Table 1 Total hydrocarbons in the initial and final samples of C soil (%, *w*/*w*)

Box	Initial sample	Final sample	Reduction (%)
2	2.64	0.041	98.45
6	1.81	0.025	98.60
7	4.14	0.045	98.92
1	1.75	0.032	98.17
8	2.98	0.043	98.55
12	4.36	0.001	99.97

was used for 1.0 g of the C soil or plant sample. At the end of extraction, the volume was reduced to 0.5 mL in a volumetric flask and evaluated via chromatography with a GC/MSD, model Shimadzu QP 5050A. The conditions used are described below:

$$\mathrm{HP}-5\mathrm{COLUMN}(60m\times 0.25mm\times 0.32\mu m)$$
$$120^{\circ}\mathrm{C}(2\,\mathrm{min})-5^{\circ}\mathrm{C}/\,\mathrm{min}-280^{\circ}\mathrm{C}(5\,\mathrm{min})$$

flow 1.0 mL/min; SCAN and SIM modes (ions =128, 152, 154, 166, 178, 202, 228, 252, 276, and 278), injected volume =1.0 μL, injection mode = split (1:20)

2.4 Quantification of Total Hydrocarbons

Total hydrocarbons can be quantified by fractionation into separate constituents of the oil mixture or extracted from a sample and detected by a particular technique. In this study, total hydrocarbons present in C soil were quantified by the sum of animal/vegetable oils and greases and mineral oils and greases according to Standard Methods 5520 (1997). The C and UC soil samples were analyzed at the beginning of the study (before planting of willow species) and at the end of the study (36 months after planting). Analyses of the total hydrocarbons in the willow species were not conducted for this study because there is no available analytical methodology.

Table 2 Quantification of PAHs in C soil before planting

Compound	Concentration (μg kg^{-1})
Pyrene	23.06
Chrysene	126.27
Benzo[*k*]fluoranthene	28.44
Benzo[*a*]pyrene	3.82

2.5 Evaluation of Plant Growth and Statistical Treatment

A caliper was used for the measurements of stem diameter, stem apex diameter, and length and width of the leaves. A tape measure was used to measure the height of the plant. The base diameter of the stem was measured at a point 2 cm above the ground, and the diameter of the stem apex was measured at a point three nodes below the apical bud. The height of the plant was measured from ground level to the apex. In each box, ten random plants were measured. After the measurements, the Q test was used to discard results, and then the means of the stem base diameter, apex stem diameter, plant height and length, and width of the leaves were calculated. The formula for the Q test is shown below.

$$Q_{\exp}=\frac{\left|X_{\mathrm{q}}-X_{\mathrm{n}}\right|}{W},$$

where:

X_{q} questioned result
X_{n} "neighboring" result
W spread of the data set

A confidence interval of 95 % was adopted. Student'st test was used to compare plant growth in UC soil (control) and C soil. The Student'st test is used both to express confidence intervals and to compare results from different experiments. The formula for Student'st test is shown below.

$$t=\frac{\overline{X}-\overline{Y}}{\sqrt{\frac{SD^2{}_x}{n_x}+\frac{SD^2{}_y}{n_y}}},$$

Table 3 Results of the PAHs analyses (μg kg^{-1}) in the six boxes containing C soil at the end of the experiment

Compound	Box					
	1	2	6	7	8	12
Phenanthrene	ND	ND	0.1	ND	ND	0.1
Pyrene	0.1	ND	0.4	ND	ND	ND
Fluorene	ND	ND	0.1	ND	ND	ND
Benzo[*a*]anthracene	0.1	ND	0.4	ND	ND	ND
Benzo[*k*]anthracene	ND	ND	ND	ND	ND	ND
Chrysene	0.1	ND	ND	ND	ND	ND

Table 4 Values of PAH analyses in leaves performed at the end of the experiment, in plants grown in C soil

Compound	Box					
	1	2	6	7	8	12
Phenanthrene	0.01	0.58	0.01	0.24	0.61	1.25
Pyrene	ND	ND	ND	ND	ND	0.09

where:

X average of the measurements
Y average of the true value
SD_x^2 standard deviation of the average of measurements
SD_y^2 standard deviation of the average of true values
N number of observations

A confidence interval of 95 % was adopted.

3 Results and Discussion

3.1 Total Hydrocarbons

The soils inside of 12 boxes were examined. Total hydrocarbons were not detected in the analysis of initial and final samples of UC soil (boxes 3, 4, 5, 9, 10, and 11). Table 1 shows the results for total hydrocarbons in the initial and final samples to six boxes containing C soil, as well as the percentage of total hydrocarbon reduction.

According to the values shown in Table 1, both species *S. triandra* and *S. rubens* reduced the total hydrocarbons concentration in C soil by more than 98 %. However, it can be inferred that the reduction in total hydrocarbons could be attributed to the physical and chemical actions of the microorganisms Silva and Souza (2005). Considering these results, it is possible to suppose that the plants were crucial in the process.

3.2 PAHs

3.2.1 Soil

In the C soil, the compounds pyrene, chrysene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene were detected. Table 2 shows the PAHs analyses at the beginning of the project (before planting). PAHs compounds in the UC soil were not detected.

Table 3 shows the results of the PAHs in the C soil at the end of the experiment (3 years after planting). PAHs compounds in the UC soil were not detected.

Comparing the initial soil analysis with the final analysis, it can be concluded that there was a visible reduction of PAHs, especially in pyrene (23.06 mg kg^{-1} at the beginning of the project to 0.1 mg kg^{-1} at the end of the project) and chrysene (126.27 to 0.1 mg kg^{-1}). The reduction of these substances through the plant is called phytodegradation. According to Dinardi et al. (2003), this process probably occurs when organic contaminants are degraded or mineralized into plant cells by specific enzymes. According to Ma and Kingscott (1997), in some cases, pollutants become small molecules that are used for plant growth. Some plants contain enzymes that break down organic substances, such as trichloroethylene and herbicides, and their fragments are incorporated to the use of the plant.

According to the results in Table 3, the box 6 (in which *S. rubens* willow was planted) showed lowest reduction of the compounds pyrene and benzo[*a*]anthracene. The plants inside this box were the ones most susceptible to attack by pests and leaf diseases, for reasons not identified during the development of the experiments.

3.2.2 Willow Species

Plants from all boxes were sampled and analyzed 18 months after planting. Roots, leaves, and stems of

Table 5 Some statistics for the stems of the species *S. rubens* in UC soil (boxes 3, 4, and 10) and C soil

Average (cm)	Box					
	3	4	10	2	6	7
Stem base (ϕ)	2.76±0.23	3.33±0.36	3.07±0.59	2.07±0.14	2.20±0.22	2.16±0.02
Stem apex (ϕ)	0.60±0.24	0.62±0.14	0.53±0.17	0.37±0.12	0.41±0.13	0.45±0.23
Stem height	181±0.06	177±0.11	174±0.06	149±0.08	167±0.07	172±0.10

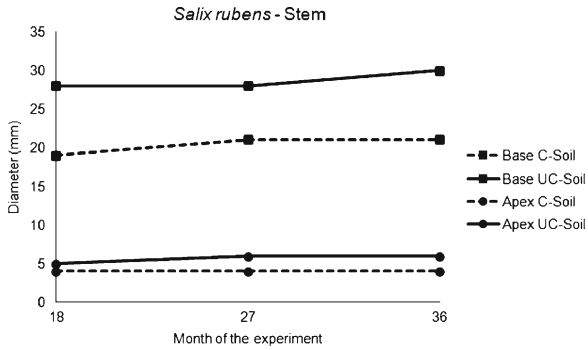

Fig. 2 Growth of both diameter at the stem base and diameter of the stem apex of the species *S. rubens*

plants from C soil boxes and leaves of plants from UC soil boxes were collected. The roots of the species *S. rubens* contained traces (ng kg^{-1}) of benzo[*a*]pyrene. No PAH compound was detected in roots of the species *S. triandra*. The leaves of the species *S. rubens* collected from C Soil contained traces (ng kg^{-1}) of phenanthrene, fluoranthene, benzo[*b*]fluoranthene, pyrene, benzo[*a*]pyrene, and benzo[*g*,*h*,*i*]perylene. The leaves of the species *S. triandra* collected from C soil contained traces (ng kg^{-1}) of fluoranthene and pyrene. The leaves of both *S. rubens* and *S. triandra* species collected from UC soil contained traces (ng kg^{-1}) of fluoranthene and benzo[*b*]fluoranthene. The results for the stems were not considered in this study due to problems in the analysis. Due to difficulties during the grinding of the stems, the results were inconsistent.

According to Ferraz (2005), with the exception of benzo[*a*]pyrene, other PAHs can be indicators of vehicular traffic, including diesel and gasoline vehicle emissions. The experiment was conducted at multiple sites, including near a truck stop (diesel fuel), an oil refinery, and a nearby highway with a heavy flow of gasoline

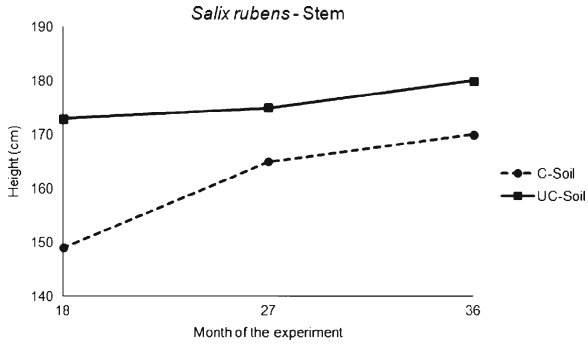

Fig. 3 Plant height evolution of the species *S. rubens* in the last 18 months of the experiment

Fig. 4 *S. rubens* grown in C soil

vehicles, especially in the early morning and late afternoon. Considering that phenanthrene, fluoranthene, pyrene, benzo[*b*]fluoranthene, and benzo[*g*,*h*,*i*]perylene were not found in the leaves or roots, it was concluded that their concentration resulted from air pollution in the surroundings. Benzo[*a*]pyrene was detected in the leaves and roots of the *S. rubens* species, which may be related to the phytoremediation process.

The last sampling of plants was held at the end of the project. Leaves and roots of plants in 12 boxes of the experiment were collected. With respect to the leaves, both phenanthrene and pyrene compounds in plants grown in C soil were detected (Table 4). The other PAHs compounds were not detected at microgram per kilogram concentration level. No PAHs compounds were detected in leaves of plants grown in UC soil. In the roots, no PAHs compounds were detected in plants at microgram per kilogram concentration level, in the 12 boxes.

3.3 Statistical Evaluation of Plant Growth

Plant growth was also evaluated over a period of 18 months (from 18th till 36th month of the experiment) by the average plant height, average diameter at the stem base, and average diameter of the stem apex.

Fig. 5 *S. rubens* grown in UC soil

Table 6 Some statistics for the stems of the species *S. triandra* in UC soil (boxes 5, 9, and 11) and C soil

Average (cm)	Box					
	5	9	11	1	8	12
Stem base (ϕ)	2.89±0.22	2.68±0.22	2.57±0.26	2.13±0.24	2.5±0.27	2.34±0.29
Stem apex (ϕ)	0.67±0.29	0.44±0.08	0.49±0.12	0.51±0.06	0.41±0.86	0.47±0.08
Stem height	182±0.04	175±0.01	181±0.06	172±0.08	184±0.04	186±0.02

The measured values were validated via statistical tests. Only two measurements of length and width of the leaves were made due to the dormancy period of the plants.

Table 5 shows a comparison of the average diameter of the stem, the diameter of the stem apex, and the average height of the species *S. rubens* grown in both UC and C soils, with the associated average type A expanded uncertainty. The plant growth was evaluated by the average plant height, average diameter at the stem base, and average diameter of the stem apex. The plants, which grown in UC soil, were used as a control. Student's *t* test was applied in the analysis of the two data sets. For the three parameters studied, the results indicated that the contaminants in soil affected the growth of the species *S. rubens*.

Figure 2 shows the progress of growth of the species *S. rubens* in terms of diameter at the stem base and diameter of the stem apex, in both UC and C soils, during the last 18 months of the experiment. Figure 3 shows the progress of the species *S. rubens* in terms of plant's height, in both UC and C soils, during the last 18 months of the experiment.

Figures 4 and 5 show plants of species *S. rubens* grown in C and UC soils, respectively. The contaminated soil decreases both the development and quality of the leaves.

Table 6 shows a comparison of the means of the stem base diameter, stem apex diameter, and plant height of the species *S. triandra* grown in uncontaminated and contaminated soils, with its associated type A expanded uncertainties, measured in the last 18 months of the experiment. Statistical analysis for the species *S. triandra* was also used for the species *S. rubens*.

Figures 6 and 7 show plants of species *S. triandra* grown in C and UC soils, respectively. As occurred with the species *S. rubens*, the leaves of the species *S. triandra* also had not a good development and quality in contaminated soil.

4 Conclusion

The two willows species *S. rubens* and *S. triandra* appeared to be resistant during the course of the project. These plants were planted in different climatic conditions from which they are typically grown and in a period not suitable for planting (off season). Plants grown in UC soil showed greater development which was verified by evaluating plant growth and an additional statistical analysis.

The reduction of soil hydrocarbons showed excellent results. There was a medium reduction of 98.56 % in total

Fig. 6 *S. triandra* grown in C soil

Fig. 7 *S. triandra* grown in UC soil

for *S. rubens* and 98.65 % for *S. triandra*. The PAHs also showed a good reduction in soil. Pyrene showed an initial concentration of 23.06 μg kg^{-1}, decreasing in most cases to 0.1 μg kg^{-1} or below detectable levels, except for box 6, which showed a concentration of 0.4 μg kg^{-1} at the end of the project. Chrysene concentrations decreased from 126.27 μg kg^{-1} early in the project to undetectable levels. Benzo[*k*]fluoranthene and benzo[*a*]pyrene also showed decreases in concentrations of 28.44 and 3.82 μg kg^{-1}, respectively, to undetectable levels. Both species are effective at hydrocarbon phytoremediation, probably due phytodegradation process, in which organic contaminants have been degraded or mineralized by specific enzymes within plant cells. However, the species *S. rubens* was more affected by contaminants, with less development of the plants grown in contaminated soil (after statistical evaluation). Even with this restriction (requiring greater care), the species *S. rubens* is indicated for the phytoremediation of hydrocarbon contaminated soils (the displayed results were very close to the species *S. triandra*) because it is a native species.

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