



Concentration of Polycyclic Aromatic Hydrocarbon (PAH) in roots and

leaves of Polyscias fruticosa used in phytoextraction of crude oil spiked soil

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Soil spiked with crude oil was successfully investigated by phytoextraction. The study was carried out to assess the potential of Polyscias fruticosa in the uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) in phytoextraction of soil spiked with crude oil. The same treatment of soil with crude oil was carried out, while the plant harvesting period was varied. Hundreds of healthy cuttings of P. fruticosa were allowed to propagate, then after 17 days, plants were transplanted in poly bags containing 500 grams of spiked soil. A set of unexposed plants were established on each harvesting period. The duration of the study was fixed for 10 months. Plant biomass of the treated and control plants were determined at the beginning and the end of the experiment. Hydrocarbons were extracted and analysed by GC-MS. The results indicated that the plant absorbed and translocated a significant concentration of PAHs. High absorption occurred on phenanthrene (194.20 mg/kg) and anthracene (208.99 mg/kg) in the root on 10 months harvesting period. Percentage removal indicates a significant reduction of PAHs from exposed soil. However, the Bioconcentration Factor (BCF) and Translocation Factor (TF) values obtained on the harvesting periods were less than 1, which indicate low absorption and translocation capacity of the plant. The study showed that P. fruticosa can be used for the extraction and translocation of PAHs from crude oil spiked soil. KEYWORDS: Polyscias fruticosa, crude oil, spiked soil, phytoextraction, Gas

chromatography

INTRODUCTION

Industrialization increases the release of chemicals into the environment which contaminate soil, water and air, these contaminants include poly cyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons (PHCs), pesticides, salts, halogenated hydrocarbons, metals and solvents. PAHs have been identified as hazardous chemicals by different State and Central Pollution Control Boards, because of their toxic, carcinogenic and mutagenic effects on living body [1]. PAHs enter and accumulate in the soil rich with organic matter which will remain for many years due their persistence to and hydrophobicity. The most common sources of PAHs in plants mentioned are (a) uptake from soil to the root, then transfer from root to the shoot (b) uptake of volatile compounds from the air by the roots or shoot of the plant [2]. Aromatic hydrocarbons are considered to be the most acute toxic component of petroleum products and are also associated with chronic and carcinogenic effects. Hydrocarbon compounds like benzene, toluene, and xylene, can affect the human central nervous system [3]. Furthermore, benzene and other petroleum hydrocarbon compounds or products, such as benzo [a] pyrene and gasoline, are considered to be carcinogenic to human beings [4]. P. fruticosa which is called Aralia in English, is from the family Araliaceae, and it is available worldwide especially in South-Eastern Asia, some countries of the Pacific region and African countries. It was reported that it needs a medium humidity and a temperature of between 16 - 29 °C to grow well [5]. There are no enough studies on this plant regarding phytoextraction of crude oil-contaminated soil. The present study aimed at assessing the potential of P. fruticosa in the phytoextraction of soil

spiked with crude oil. The objectives of this study were (a) to assess the ability of *P. fruticosa* to survive on a soil spiked with crude oil (b) and to evaluate the uptake of PAHs by the root and its transfer to the leaves and (c) compare results with the control plant and soil. The contribution of this study is the capability of *P. fruticosa* to survive on crude oil contaminated soil, uptake, accumulate and transfer polycyclic aromatic hydrocarbons to above-ground tissues in a greenhouse condition. The outcome of the research shows that P. fruticosa was effective and efficient to accumulate PAHs from crude oil spiked soil.

MATERIALS AND METHODS Soil history

The soil used in this study was collected from the Serian district located about 59 km from Kuching city, Malaysia. The soil sample called serin serry which is red in colour (dominant on top soil), probably caused by truncation as a result of erosion [6]. The site was selected randomly and the establishment of a grid in soil sampling was not considered. Several physical and chemical characteristics of the serin serry soil was determined before being used in the study.

Soil parameters

The physical and chemical analysis of soil samples collected was conducted before and after spiking the soil with crude oil. The pH of the soil was tested according to the procedure explained by Farahat et al [7], while electrical conductivity (EC) was conducted as described by Rayment and Higginson [8]. Moisture, organic matter, and ash contents were determined by following the procedure described in the American Society for Testing and Materials (ASTM) standard methods [9]. Total organic carbon (TOC) of the soil was determined according to the procedure outlined by Nelson and Sommers [10].

Plant Sample

Healthy *P. fruticosa* cuttings were transplanted into polybags containing spiked soil. Prior to this, the cuttings were allowed to propagate freely for 17 days in a growth chamber. Sufficient number of polybags (200) were prepared and maintained in the green house.



Figure 1: The picture of *P. fruticosa* plant used in this research

Crude oil

The crude oil was obtained from the Baram oil field at the offshore of Miri, Sarawak. The sample was extracted from underground by Sarawak shell Berhad Malaysia and consist of a mixture of AHs and PAHs. A stock solution of 1000 μ g/mL was prepared by weighing 100 mg of crude oil and diluted it to 100 mL mark of 100 mL volumetric flask with distilled water. A working solution 50 µg/mL was prepared by using the formula $C_1V_1 =$ C_2V_2 where, C_1 is the concentration of stock solution to be used, C₂ is the concentration of working solution to prepare, V_1 is the volume of stock solution to be used and V_2 is the volume of working solution need to prepare. A crude oil solution of 50 µg/mL was used to spike into each 500 grams of soil and mixed thoroughly in a polybag. The polybags were stored in a dark fume hood for 7 days for solvent evaporation and equilibration to take place. A set of control soil (unexposed soil) were established for each harvesting period at the greenhouse of Faculty of Resource Science and Technology (FRST), N 01° 33' 03.6" E 110° 45' 56.6" of Universiti Malaysia Sarawak (UNIMAS). The concentration of PAHs in crude oil, uncontaminated soil, and spiked soil was determined using gas chromatographymass spectrometer (GC-MS).

Pot experiment

Polybags were arranged in the greenhouse which was netted to minimize rain water leaching, and covered with nylon to prevent insects and birds. Healthy young plants with approximately the same height (4 cm) were selected for the study. All pots were kept in the green house at 22 - 25 °C (with a daily minimum average of 19 °C to maximum 35 °C). A partially decomposed and dried organic manure (250 mg kg⁻¹ soil) was used to improve the quality of the soil (exposed and unexposed) samples. The same quantity of manure was added to each pot.

Plant and soil analysis

The plants were harvested after 2, 4-, 6-, 8-, and 10-months of harvesting periods [11]. The extraction of PAHs from the soil, roots and leaves was carried out according to Zhang et al. [12], with some modification in the weight of samples, amount of time solvent and of extraction. Approximately 5.0 grams of plant part was extracted (Soxhlet extraction) with 250 mL of dichloromethane for 8 hours. Dichloromethane was evaporated to nearly 1 mL using a vacuum rotary evaporator to obtain crude extract. The crude extract was fractionated on a silica gel column chromatography according to the procedure described by El Nemr et al. [13]. A small glass column chromatography was

packed with 5.0 g activated silica gel (230-400 mesh) and used to obtain F1 and F2 fractions. The crude extract was diluted with 1 mL of *n*-hexane and placed on the top of the silica gel layer in a column. F1 and F2 fractions were subsequently isolated by eluting with 40 mL n-hexane and 40 mL mixture of n-hexane and dichloromethane (1:1, v/v), respectively. Each F1 and F2 fractions were collected in 100 mL pear-shaped flask and a evaporated to almost 1 mL using a vacuum rotary evaporator. Both F1 and F2 fractions 1 then dissolved in mL were dichloromethane. sonicated. and transferred to 5 mL vial using Pasteur pipette. Both fractions were then gently evaporated to dryness using purified nitrogen gas and kept in a dark place at 4 ⁰C temperature until further analysis using GC-MS. The PAHs fractions analysed were, naphthalene (Nap), acenaphthylene (Acy) acenaphthene (Ace), fluorene (Flu), Phe, anthracene (Ant), Pyr, chrysene (Chry), fluoranthene (FluA). benzo[a]anthracene (BaA), dibenz[a,h]anthracene (DBA), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF), benzo[b]fluoranthene (BbF), benzo[g,h,i]perylene (BP). and indenol[1,2,3-cd]pyrene (IP) performed on a Shimadzu Gas Chromatography-Mass Spectrometer **QP2010** Plus model equipped with quadrupole mass analyser. Prior to GC-MS analysis, fraction F2 was dissolved in 500 µL using dichloromethane (GC grade). Identification and quantitation of PAH components were performed by direct comparison of retention times of individual PAHs in a mixture of PAHs standard. The same procedure was followed for the extraction, fractionation, and analysis of control spiked soil.

Removal of Elemental Sulphur

The presence of sulphur interferes with the analysis of hydrocarbons through the process of

absorption which affects the molecular weight. At an elevated temperature, the reaction between sulphur and hydrocarbons yield a hydrogen sulphide and dehydrogenated compounds.

A glass column was prepared with a bed of activated copper powder to treat the PAHs fraction. The PAHs sample fraction was diluted with 20 mL dichloromethane and eluted slowly down the column [14].

Table 1: physical and chemical p	(n=3)	
Parameters	Control soil	Spiked soil
рН	6.9±0.12	6.1±0.09
Electrical conductivity µs/cm	197±0.09	165±0.36
Organic matter (%)	2.68	3.94

4.87

Analysis of data

The results presented is the mean and standard deviation of 3 replicate of plant sample. Bioconcentration factor (BCF) was determined according to BCF = pp/ps, where pp = PAHs in plant root, ps = PAHs in spiked soil. Translocation factor (TF) was determined according to TF = pps/ppr,

Total organic carbon (%)

RESULTS AND DISCUSSION

The characteristics of control and spiked soil samples were presented in Table 1. The pH of spiked soil samples was more acidic compared to the control soil. The pH increase of spiked soil could be due to where pps = PAHs in plant shoot and ppr = PAHs in plant root. The significance was set at $P \le 0.05$ (5%).

6.19

Statistical analysis

Pearson correlation coefficient for unexposed soil and exposed soil was calculated using excel 2019.

generation of acidic intermediates during decomposition of organic compounds that are present in crude oil [14]. The electrical conductivity of spiked soil was low when compared to the control soil sample. Osuji and Nwoye [15], reported a decrease in electrical conductivity of spiked soil compared to controls. The total organic matter (TOC) and organic matter content (OMC) were high in the spiked soil when compared to the control soil samples. The high level of TOC and OMC in spiked soil could be due to high number of carbon compounds present in crude oil. The

concentrations presented are the values of PAHs expressed on a dry-weight basis of soil and *P. fruticosa* tissues (oven-dried at 105 0 C for 24 h) and mean of 3 replicate extractions. Table 2 shows the initial concentration of PAHs in the control soil sample (uncontaminated soil), spiked soil and concentration of PAHs in *P. fruticosa*.

Table 2: Mean Concentration (mg/kg) of PAHs in uncontaminated soil,

P. fruticosa and spiked soil (n=3)				
Compound Name	P. fruticosa	Uncontaminated soil	Spiked soil	
Nap 2	Nd	0.02±0.01	308.52±13.52	
Acy 2	0.79±0.05	0.09±0.03	437.09±21.17	
Ace 3	0.07±0.01	0.07±0.02	693.58±17.39	
Flu 3	0.25±0.04	0.15±0.06	1883.24±26.77	
Phe 3	4.13±0.96	0.13±0.04	297.56±17.03	
Ant 3	13.05±2.43	0.05±0.01	1992.38±32.64	
FluA 4	10.64±1.27	0.04±0.02	1514.58±29.33	
Pyr 4	3.44±0.76	0.14±0.60	1382.24±24.68	
BaA 4	0.37±0.08	0.07±0.01	1094.21±36.39	
Chy 4	9.41±1.03	0.11±0.03	609.64±19.67	
BbF 5	5.36±0.61	0.06±0.01	494.82±26.83	
BkF 5	3.57±0.12	0.07±0.04	825.69±42.07	
BaP 5	Nd	0.03±0.01	1020.63±50.36	
DahA 5	0.19±0.01	0.09±0.07	801.31±37.51	
BghiP 6	4.67±0.77	0.07±0.03	933.84±34.20	
IcdP 6	3.62±0.15	0.02±0.01	1153.62±48.35	
The Table shows the initial concentration of PAHs in <i>P. fruticosa</i> plant, uncontaminated soil and spiked soil, nd = not determined.				

Plant growth and biomass

P. fruticosa showed a good response when planted on soil spiked with crude oil. The plant has high tolerance and adaptability to the condition. It was suggested that the spiked soil have the strength to promote plant growth since it contains organic manure or it has the strength to support the organism that is responsible for promoting the growth of the plant. These organisms give rise to plant growth prerequisites, supply nitrogen, and protect it from other effects [13].

Absorption of PAHs by plant root

Table 3: PAHs concentration (mg/kg) in the root of *P. fruticosa* grown on crude oil contaminated soil at different harvesting period and concentration of PAHs in the root of unexposed *P. fruticosa*

Compound	2	2 months	4	4 months	6	6 months	8	8 months	10	10 months
Name	months	control	months	control	months	control	months	control	months	control
Nap 2	nd	nd	1.02±0.35	nd	16.78±1.53	nd	34.15±2.14	nd	72.56±2.38	nd
Acy 2	2.45±0.51	0.57±0.05	7.36±1.21	0.40 ± 0.04	67.76±3.78	0.37±0.06	93.84±3.57	0.44±0.07	114.00±4.67	0.32±0.01
Ace 3	0.24±0.01	0.06±0.01	0.57±0.01	0.08±0.01	11.73±1.24	0.12±0.02	64.32±1.38	0.03±0.00	84.44±2.15	nd
Flu 3	0.67 ± 0.01	0.19±0.04	4.47±0.39	0.17±0.03	87.48±2.68	0.28±0.05	125.65±2.69	0.02±0.03	158.27±6.71	nd
Phe 3	12.19±2.46	3.11±0.96	38.81±2.42	3.22±0.98	74.00±1.69	1.35±0.93	127.7±1.35	0.94±0.12	194.20±2.59	0.07±0.01
Ant 3	38.99±1.87	10.08±2.43	80.98±3.68	10.91±2.53	133.09±8.26	8.29±2.47	176.4±3.97	6.35±1.31	208.99±5.37	5.14±1.23
FluA 4	32.86±3.16	9.49±1.27	36.79±2.52	11.13±1.28	51.05±1.36	7.28±1.23	83.34±4.25	5.34±0.16	111.26±1.25	4.10±0.17
Pyr 4	10.31±1.05	3.11±0.76	16.22±1.34	3.03±0.56	36.25±1.28	2.67±0.56	69.64±5.02	2.04±0.13	93.81±3.20	1.15±0.08
BaA 4	0.99±0.13	0.32±0.05	10.7±1.11	0.28±0.08	44.98±2.47	1.01±0.09	89.66±2.36	0.90±0.07	141.00±1.99	0.02±0.01
Chy 4	28.13±1.52	9.00±0.73	30.16±2.46	7.52±1.03	47.081.31	5.71±1.04	78.63±1.89	3.11±0.04	101.96±2.47	2.67±1.16
BbF 5	15.99±1.24	5.12±0.61	24.15±2.07	3.24±0.51	36.94±0.96	2.97±0.65	50.15±2.71	2.43±0.32	68.53±1.38	2.31±0.54
BkF 5	11.33±1.03	3.06±0.18	65.82±2.89	2.68±0.13	92.61±3.81	1.79±0.13	127.37±6.97	1.27±0.21	146.22±2.05	1.09±0.27
BaP 5	nd	nd	1.70±0.36	nd	16.84±1.02	nd	37.98±1.23	nd	53.50±1.32	nd
DahA 5	0.58±0.02	0.21±0.01	9.13±1.04	0.10±0.01	15.16±0.65	0.08±0.01	32.65±1.40	0.03±0.00	61.71±1.41	nd
BghiP 6	14.01±1.38	4.67±0.34	37.06±2.12	2.73±0.36	54.53±0.21	2.02±0.76	88.71±2.51	1.62±0.47	135.62±1.36	1.28±0.35
IcdP 6	10.36±1.74	3.46±0.11	41.42±1.58	2.02±0.14	117.77±3.42	2.15±0.15	138.31±6.34	1.85±0.31	173.27±2.58	1.19±0.26

Most of the target PAHs in the crude oil contaminated soil were detected in the root of the plant. A high absorption occurred for anthracene, phenanthrene, and indeno [1,2,3-c,d] pyrene in the root on 10 months with concentrations 208.99, 194.20 and 173.27 mg/kg, respectively (Table 3). The increase in PAHs concentration in the root may be due to the ability of the plant to extract hydrocarbons from the soil. The dispersion of high molecular weight PAHs is less due to their bulky structure [15]. The occurrence of fluorene in plant roots indicates the transfer of PAHs from spiked soil to the plant's root and subsequently bioaccumulate in plant parts.

Translocation of PAHs to plant leaves

Table 4 shows the occurrence of PAHs with their concentrations translocated to the leaf of the plant. The most abundant PAHs in the leaf on 2 months was chrysene with a concentration of 2.04 mg/kg. Naphthalene, acenaphthylene, and acenaphthene the had lowest concentrations of 0. 07, 0. 05, and 0.03 mg/kg, respectively in 2 months. An increase in PAHs translocation to the leaf was significant on 6, 8, and 10 months. Hydrocarbons of Nap, Ace, Acy, fle, BaP, and DahA were not detected from unexposed (control) plant leaf, however, hydrocarbon BaA was detected. The

presence of some PAHs in unexposed plant and its subsequent disappearance on the harvesting period, could be due to degradation or their conversion to some other metabolic intermediates within the harvesting period [16].



Figure 2: The GC-MS chromatogram of the PAHs from the root of *P. fruticosa* plant on 2-months harvesting period.



Figure 3: The GC-MS chromatogram of the PAHs from the root of *P. fruticosa* plant on 4-months harvesting period.



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Figure 4: The GC-MS chromatogram of the PAHs from the root of *P. fruticosa* plant on 6-months harvesting period. Unresolved issues were resolved later.



Figure 5: The GC-MS chromatogram of the PAHs from the root of *P. fruticosa* plant on 8 months harvesting period. Unresolved issues were resolved later.



Figure 6: The GC-MS chromatogram of the PAHs from the root of *P. fruticosa* plant on 10-months harvesting period. Unresolved issues were resolved later.

The disappearance of few PAHs in the leaf's samples, which appeared in the roots, could be due to improper translocation of PAHs through the plant

Concentration of PAHs in plant parts

The concentrations and composition of sixteen PAHs in the roots and leaves of *P*. *fruticosa* were illustrated in Table 3 and Table 4, respectively. This research clearly

and may be due to high adsorption of the organic substances by the hydrophobic lipid components of plant root [17].

indicates that *P. fruticosa* can absorb and retains the PAHs within its roots with little translocation to leaves. In a similar study conducted on rice, Wang et al. [18] recorded a high concentration of PAHs in the root and little transfer to the shoot. Contrary to this study, it was reported that four mangrove species (*Kandeliaobovata*, *Avicenniacorniculatum*, *B. gymnorrhiza*, and *Avicennia marina*) grown on crude oil spiked soil in mangrove swamps in China, accumulated a significant quantity of PAHs in the leaf higher than that in the root [18]. In general, PAHs with low molecular weight (LMW) was extracted and transferred to the aboveground tissues while high molecular weight (HMW) is stored within the plant roots [19].

Low molecular weight PAHs appeared in high quantity in plant organs due to their high-water solubility, vapor pressure, and availability [20]. In the roots of P. fruticosa, LMW PAHs compounds accumulated include acenaphthylene (114.00) acenaphthene (84.44), fluorene (158.27) and naphthalene (72.56), and those with 3 rings include phenanthrene (194.20) and anthracene (208.99), in mg/kg, on 10 months harvesting period.

Anthracene was found in high abundance on 10 months of harvesting period, which may be attached strongly with the cell-wall components like lignin, pectin, cellulose, and hemicellulose [21] and this could be the reason for its abundance (208.99 mg/kg) in the root. The results show that PAHs with higher ring numbers were absorbed more in the root and a little was translocated. It could be due to the 2 major entrance of organic pollutants into plants include: (a) absorption of PAHs by plant root from the soil and (b) uptake of PAHs from the atmosphere and deposited on the leaves through stomata [12]. Bkf and BghiP with 5 and 6 rings respectively, were found to be high on the 10 months harvesting period. The quantity of PAHs in the leaves of unexposed plant were less compared to the exposed plant. High molecular weight PAHs are mostly and strongly accumulated in soil organic matter and on the plant root, which may possibly stop them from being carried away by the conducting tissue [20].

PAHs	Spiked Unexposed (control) soil	Spiked Exposed soil	R%
Nap 2	119.7	nd	61.2
Acy 2	124.81	0.32	71.45
Ace 3	163.76	nd	76.39
Flu 3	992.58	0	47.29
Phe 3	86.35	0.07	70.98
Ant 3	1420.11	5.14	28.72
FluA 4	926.27	4.1	38.84
Pyr 4	854.33	1.15	38.19
BaA 4	897.62	0.02	17.97
Chy 4	473.48	2.67	22.34
BbF 5	359.65	2.31	27.32

Table 5: PAHs concentration (mg/kg) in spiked unexposedsoil, spiked exposed soil and the percentage removal

BkF 5	537.23	1.09	34.94	
BaP 5	806.11	nd	21.02	
DahA 5	639.67	nd	20.17	
BghiP 6	728.42	1.28	21.99	
IcdP 6	947.19	1.19	17.89	
The Table shows the concentration of PAHs in spiked unexposed soil, spiked soil and the percentage removal				

Table 5 shows the final concentration of PAHs in spiked unexposed soil, spiked soil and the percentage removal. There is a reduction in the concentration of PAHs in spiked unexposed soil which may be due to volatility of PAHs. Hydrocarbons of Nap, Ace, Flu, BaP, and DahA disappeared from spiked exposed soil at the end of the study. The hydrocarbons of Ace have the highest percentage removal, while IcdP has the lowest percentage removal.

The Pearson correlation coefficient for unexposed soil and exposed soil was calculated using Excel 2019 office, and it shows a perfectly negative correlation between the variables. The degree of freedom (DF) was 14, and 2 tail tests was used which shows the p-value to be 0.003. The coefficient value R = -0.66, indicate that there is no significant correlation between them.

Bioconcentration factor and translocation factor

The BCF values were not consistent, but the values on 10 months harvesting period was about two times greater than those on 2-, 4-, and 6-months harvesting periods. If BCF is greater than 1, the plant has the capability to uptake and translocate the heavy metals. The data presented in Figure 3 (a and b) show the values of bioconcentration and translocation factors of PAHs. The *P. fruticosa* has the ability to absorb and transfer PAHs to the aboveground parts, with low BCF and TF, therefore, this plant is not very sufficient for extraction. The translocation factors PAHs revealed that the were not effectively and efficiently transferred to the above-ground parts of the plant since its accumulation ratio was < 1 TF (Figure 3). "It has been shown that plants growing on PAH-contaminated soils may contain PAHs in their tissues which may originate from volatile compound absorption by leaves in the surrounding air, deposition of contaminated soil particles (splash), and dust on leaves, followed by retention in cuticle or penetration through it and soilto-root transfer followed by subsequent translocation by the transpiration stream" [22].



Figure 7: The bioconcentration and translocation factors of PAHs in the five different harvesting period. Both the BCF and TF were less than 1.

The uptake of PAHs from soil to root was investigated from hydroponic studies [23] spiked soils [24]. and This study demonstrated that PAHs in crude oil spiked soils can be detected at a significant amount in the root and translocated small to plant leaves. Also, the concentration of PAHs accumulated in the leaves of the plant resulted from plant root uptake and subsequent translocation from root to the leaves. However. the BCF values calculated on harvesting periods were very low which ranged between 10^{-4} to 10^{-2} .

CONCLUSION

This study demonstrated phytoremediation of plant grown in crude oil spiked soil which could be achieved by using P. fruticosa. Although the concentration of PAHs in plants can come from both the atmosphere and spiked soil. the predominant one in this study was soil to root uptake by the plant. A significant concentration of PAHs was accumulated in the roots of the plant. The leaves of the plant responded positively but with an accumulation of a small concentration of PAHs. The concentration of PAHs in the soil does not affect the growth of the plant

even at a longer harvesting period. Therefore, despite the accumulation of PAHs in the root, P. fruticosa can grow in the spiked soil without a sign of phytotoxicity and other harmful effects on biomass production. This characteristic indicates that P. fruticosa has the capability to remove **PAHs** from contaminated soil. More research should conducted to investigate be the

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phytoremediation efficiency of this plant on long term field contaminated soil.

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