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Research note

Screening for Resistant and Tolerable Plants (*Ludwigia Octovalvis* and *Phragmites Karka*) in Crude Oil Sludge for Phytoremediation of Hydrocarbons

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A B S T R A C T

Phytoremediation is a process which utilizes plants to remove contaminants from the environment. It is the latest alternative to treatment technique that needs to identify potential plants and their ability to resist toxicity of contaminants before a full scale system can be installed to ensure that the remedy is effective by the selected plants. The aim of this study is to evaluate the ability of two native plants in Malaysia, *Ludwigia octovolvis* and *Phragmites karka*, to survive when exposed to real crude oil sludge. The experiment was performed in a greenhouse for 15 days. The observation was made three times a week. The plants were also watered using tap water to ensure the plants could grow. After 15 days of observation, the two plant species had shown that they could grow and survive in pots with 100% of crude oil sludge. From this preliminary test, *L. octovolvis* and *P. karka* showed their initial ability to treat sand contaminated with crude oil sludge. As a conclusion, both native plants have the potential in the phytoremediation process of hydrocarbon and will be used in future prolonged phytoremediation of crude oil sludge.

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INTRODUCTION

Petroleum is one of the most important energy resources for industry, daily life and a raw material of the chemical industry. Petroleum is a complex mixture consisting of thousand compounds known as hydrocarbons [1,2]. The problems arises from petroleum exploration that is currently solved through clean up activities. Common techniques involved in the cleaning up of soil contaminated sites are the physical, chemical and thermal processes [3]. These techniques however have some adverse effects on the environment and are also expensive [3,4]. Some of the techniques are costly and not environmentally friendly leaving recalcitrant by-products in the environment. Recently, biological techniques are being evaluated for the remediation of sites contaminated with petroleum. Phytoremediation is one of the biological techniques for cleaning up polluted soils. It is a highly flexible and solar driven in situ pollutant extraction system for removal of contaminants from soil, water, sediments, and air. Phytoremediation potential has been widely accepted as highly stable and dynamic approach for reducing eco-toxic pollutants. It is also a highly perceptive and promising field of bio resources technology [5]. Among the different remediation techniques, phytoremediation is proposed to be efficient and cost-effective with high public acceptance and environmentally friendly aspects [6 ,7, 8, 9]. Comparing natural attenuation between bioaugmentation and phytoremediation, it was reported that phytoremediation was the most efficient technique for cleaning up contaminated soil [10]. Phytoremediation has been shown to be effective for petroleum and assessed in a number of field and greenhouse studies [11, 12]. Plants used for phytoremediation should have the following characteristics: (i) tolerant to high levels of the hydrocarbon, (ii) accumulate reasonably high levels of the hydrocarbon, (iii) rapid growth rate, (iv) produce reasonably high biomass in the field, and (v) have profuse root system [13]. Table 1 lists down some plants used to treat hydrocarbons in soils. The plant used in this research is *Ludwigia octovalvis*, known as Mexican primrose-willow. It is herb robust, erect, perennial, sometimes woody at base or even shrubby. *L. octovalvis* is a cosmopolitan plant with a mainly tropical distribution. The second plant used is *Phragmites karka*, a local plant and is in poaceae family. This research was carried out to screen for native plants that can resist and tolerate in crude oil sludge for phytoremediation of hydrocarbons. The growth of two native plants, *L. octovalvis* and *P. karka* were observed for their survival and resistance in real crude oil sludge. After determining their survival and tolerance, further work will be done extensively to analyze the concentration in another phytotoxicity test.

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MATERIAL AND METHODS

Propagation of plants of *Ludwigia octovalvis* and *Phragmites karka*

L. octovalvis and *P. karka* werepropagated in a greenhouse in Universiti Kebangsaan Malaysia using soil garden in polyethylene bags with composition of soil garden was 3: 2: 1 for topsoil: organic: sand. After one-month propagation, the plants were ready and transferred to the tested pots.

Source for crude oil sludge

Crude oil sludge which was used to test capability of plants to survive was obtained from a contaminated oil fields of a crude oil terminal in Malaysia. The sludge was characterized for its TPH, BTEX and nutrients content.

Total petroleum hydrocarbon extraction in soil The total petroleum hydrocarbon (TPH) in the samples was analyzed using an ultrasonic solvent extraction method [1]. Three replicates of spiked medium were sampled at each sampling periods. The collected samples were stored in glass bottles and kept at 4 °C prior to analysis. Approximately 10 g of each sample was placed in a 100-mL flask from each pot on the same sampling day for all treatments to extract TPH. Soil samples were dried by mixing with sodium sulfate (Na_2SO_4) and later placed in a 100-mL Schott bottle with 50 mL dichloromethane (DCM) (R&M Chemicals, UK), with the bottle being agitated in an Ultrasonic cleaner (Thermo-10D, USA) for 30 min at 50 °C. The supernatant was filtered through glass wool. The extracts were concentrated and were left in the fume hood for 2–3 days to allow the solvent to evaporate completely, after which, 1.5 mL DCM was added and the extracts were stored in gas chromatography vials.

Experimental set-up for plant screening

The experimental set-up for plant screening was prepared as depicted in Table 1. Three plants were grown in each 3 L yellow pot containing 3 kg of the crude oil sludge. Two replicates were used for each plant. Another pot containing plants in 3 kg sand acted as a plant control (PC). The observation was conducted three times a week for 15 days for each plant by physically observing the ability of the plants to survive and resist the hydrocarbon contaminant in terms of the number of withered plants within the period. The percentage of withered plant was determined by dividing thenumber of witheredplantswith the total plants.

RESULTS AND DISCUSSION

Crude oil sludge which was used to test capability of plants to survive was obtained from a contaminated oil fields of a crude oil terminal in Malaysia. The sludge was characterized for its TPH and nutrients content as summarized in Table 2.

TABLE 1 Experimental set-up for plant screening

Plant	Plants in crude oil sludge (R1)	Plants in crude oil sludge (R2)	Plants in sand (PC)
Ludwigia octovalvis			
Phragmites karka			

TABLE 2. Characteristics of crude oil sludge used for the plant screening

Parameters	Unit	Value
TPH	mg/kg	1.47E+04
BTEX	mg/kg	0.005
Ammonia (NH_3-N)	mg/kg	4.09
Nitrate (NO_2—N)	mg/kg	0.028
Nitrite (NO_3-N)	mg/kg	4.7
Phosphate (PO_4^{3-})	mg/kg	2.09
pH	-	6.7

It contained 1.47E+04 mg/kg of TPH that are hazardous to the environment. The physical growth observation of withering effects of crude oil sludge on the physical plant (withered or dried) were conducted, based on the change of colour from the normal green color to brown color and shown in Table 3.

Based on the observation, after 7 days of exposure, *L. octovalvis* plants were still healthy but for *P. karka*, one planthad begun to wither while the rest were still healthy. On Day 15 for *L. octovalvis*, two plants had withered in the second replicate pot. The rest of plants was still healthy and green in the first replicate pot. Whilst, for *P. karka*, plants were still healthy in the first replicate and one plant in the second replicate had turned its colors from green to yellow and finally died at the end of exposure. Both plants showed different survival abilities at the real crude oil sludge. Both plants in the PC pots remained green and healthy in the crude oil sludge and survived until Day 15. Figure 1 shows the survival rates for the two plants at day 15 with 66.6% survival for *L. octovalvis* and 83.3% for *P. karka*. The result of this study suggested that these plants showed a high resistance to crude oil concentration acting as a model of hydrocarbon contaminant. Exposure to gasoline can occur for workers on the manufacturing chain, from gasoline production to consumer use, gasoline at docks and gasoline fueling stations or gasoline exposure as inhalation of gasoline vapors during automobile refueling [15].

TABLE 3. Plant growth observation of on Day 0 and Day 15 for *Ludwigia octovalvis* and *Phragmites karka*

Plant	Day	Plants in crude oil sludge (R1)	Plants in crude oil sludge (R2)	Plants in sand (PC)
Ludwigia octovalvis	Day 0			
	Day 15			
Phragmites karka	Day 0			
	Day 15			

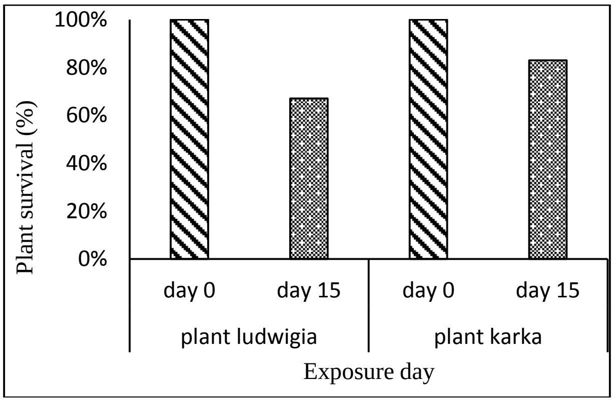

Figure 1. Percentage survival of *Ludwigia octovolvis and Phragmites karka* on Day 0 compared to Day 15

Therefore, it is expected that these two native plants will give impressive results if used to treat crude oil sludge contaminated soil due to its high ability to adapt it. Most phytoremediation research has focused on specific plant to be used in phytoremediation to enhance the degradation of organic pollutants and crude oil sludge was chosen as a model contaminant to represent petroleum hydrocarbons environment [14]. Under this screening test, the physical observation for two plants in pots with real crude oil sludge had turned its colors from green to yellow. Similar results were observed in a study by Sanusi et al. [16]. The continued growth of the plants in the presence of the hydrocarbon contaminant can be the first indication that these plants are potential phytoremediant [17]. While other researchers showed when exposed to diesel all species can survive up to 0.05 mL/L diesel (v/v) but in concentration of 0.5 mL/L, *Azolla pinnata* died due to the toxicity of high diesel concentrations [18].

Results also showed that *S. mucronatus* exposed to different levels of gasoline died after one day in high concentrations. While the concentration of 40 g gasolines/kg to 70 g gasoline/kg plants died after 7 days. However, plants can grow successfully in low concentration of gasoline in which the findings from this study also agrees with the study conducted by Purwanti et al. [19] who had observed *Scirpus mucronatus* when exposed to diesel fuel concentrations of 5, 10, 15, 100 and 200 g/kg the plants had changed their colors, from green to yellow and the plants in concentrations of 100 and 200 g/kg were withered after 10 days of exposure. Another study concluded that *Paspalum scrobiculatum* can grow in different concentration of crude oil (2.5, 5, 7.5, 10.0, 12.5 and 15.0%) and the length was significantly reduced by the presence of crude oil .

CONCLUSIONS

After15 days of observation, it was found that 66.6% of *L. octavalvis* and 83.3% *of P. karka* could survive and grow successfully inpots containing crude oil sludge. Therefore, these plants are good candidates for remediation ofhydrocarbon in contaminated crude oil sludge. Hence, the future phytotoxicity test will be conducted on 100% crude oil by analyzing in details the TPH and heavy metal removals.
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Persian Abstract

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چکیده

Phytoremediation یک روند است که از گیاهان برای حذف آلاینده ها از محیط استفاده می کند. این جدیدترین جایگزین برای تکنیک درمان است که نیاز به شناسایی گیاهان بالقوه و توانایی آنها در مقاومت در برابر سمیت آلاینده ها دارد تا یک سیستم مقیاس کامل بتواند نصب شود تا مطمئن شود که این گیاه توسط گیاهان انتخاب شده موثر است. هدف از این مطالعه ارزیابی توانایی دو گیاه بومی مالزی، *Ludwigia octovolvis* و *Phragmites karka* برای زنده ماندن در معرض لجن نفت خام واقعی است. آزمایش در گلخانه به مدت ۱۵ روز انجام شد. مشاهدات سه بار در هفته ساخته شد. گیاهان نیز با استفاده از آب شیرین آب آشامیدنی جهت اطمینان از رشد گیاهان، آب می شوند. پس از ۱۵ روز مشاهده، دو گونه گیاهی نشان داده اند که می توانند رشد کنند و در گلدان ها با ۱۰۰٪ لجن نفت خام تولید کنند. از این آزمایش اولیه، *L. octovolvis* و *P. karka* توانایی اولیه خود را برای درمان شن و ماسه آلوده به لجن نفت خام نشان دادند. به عنوان یک نتیجه گیری، هر دو گیاهان بومی دارای پتانسیل در روند فیتوراسیون از هیدروکربن هستند و در فیتوتراپی طولانی مدت بعد از لجن نفت خام استفاده خواهند شد.