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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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P A P E R I N F O

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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, phytoremediation needs to identify these plants and its ability to resist toxicity of contaminants before a full scale system can be installed to ensure that the remedy is effective by selected plants. The aim of this study is to evaluate the ability of two native plants in 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 its 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 and exploitation 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 while some are 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 versatile, solar driven in situ pollutant extraction system for removal of ecosystem trembling 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 signifies 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 plants used is *Phragmites karka*, a local plant and 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

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hydrocarbons. The growth of two native plants, *Ludwigia octovalvis* and *Phragmites karka* were observed whether they could survive after the exposure with crude oil sludge. After determining their survival and tolerance, further work will be done extensively to analyze the concentration in another phytotoxicity test.

MATERIAL AND METHODS

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

Ludwigia octovalvis (L.) and *Phragmites 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.

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. 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 the number of withered plants .

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			

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 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 environmentThe 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.

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			

Based on the observation, after 7 days of exposure, *L. octovalvis* plants were still healthy but for *P. karka*, one plants had begun to wither while the rest were still healthy.

Based on the physical observation of the plant growth 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. However for *P. karka*, plants were still healthy in the first replicate while one plant in the second replicate had turned its colors from green to yellow and finally died at the end of exposure. Both plants show 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 were 66.6% 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 and performed better compared to plant from other studies.

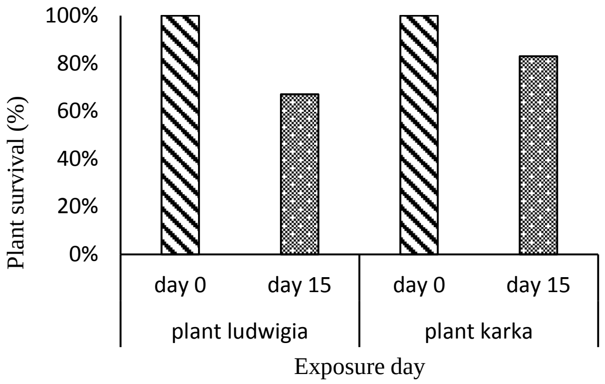

Figure 1. Percentage survival of *Ludwigia octovolvis and Phragmites karka* on Day 0 compared to Day 15

For example in the study of Azollapinnata [16] the plant showed resistance to diesel concentration only up to 0.5mL/L. which were exposed to different concentration of diesel. 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]. 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 is inhalation of gasoline vapors during automobile refueling [15]. 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 the study also agrees with the [19] observed when exposure plants *S. mucronatus* to diesel fuel concentrations of 5, 10, 15, 100 and 200 g/kg had changed their colors, from green to yellow or and plant 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 [17].

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CONCLUSION

After15 days of observation, it was found that 66.6% of *L. octavalvis* and 83.3% *of P. karka* could survive and grow successfully in pots containing crude oil sludge. Therefore, these plants are good candidates for remediation of hydrocarbon 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 آخرین روش جایگزین برای تکنیک های درمان است، گیاهان دارویی نیاز به شناسایی این گیاهان و توانایی آن در مقاومت در برابر سمیت آلاینده ها قبل از اینکه یک سیستم مقیاس کامل بتواند نصب شود تا مطمئن شود که این گیاه توسط گیاهان انتخاب شده موثر باشد. هدف از این مطالعه ارزیابی توانایی دو گیاه بومی در 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 را نشان داد. 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.