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Phytoremediation of crude oil-contaminated soil with local plant species

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Abstract. Contamination of soil by crude oil is prevailing due to increasing petroleum-related activities. Soil contaminated by crude oil is often rendered unfit for habitation and agriculture due to health concerns. Phytoremediation has emerged as a cost-effective and uncomplicated method of remediating crude oil-contaminated soil. This study examines the potential of four local plant species in phytoremediation of crude oil-contaminated soil, i.e. Pteris vittata, Epipremnum aureum, Mucuna bracteata and Imperata cylindrica. Changes in the crude oil concentrations, pH and moisture of potted soil planted with the aforementioned species were tracked over six weeks. The results were compared against an un-vegetated pot of soil serving as control. The results showed that all vegetated pots had higher crude oil removal compared to the control. Epipremmum aureum demonstrated the highest ability of crude oil removal with 50.4% of crude oil removed, followed by Imperata cylindrica (39.5%), Pteris vittata (36%) and Mucuna bracteata (30.9%). The sequence of plant species in terms of increasing rate of crude oil removal is Pteris vittata, Imperata cylindrica, Mucuna bracteata and Epipremmum aureum. Soil moisture and pH fluctuated over small ranges. This study contributes to identification of local, readily-available plant species and their effectiveness for cost-effective phytoremediation of crude oil-contaminated soil.

1. Introduction

Soil contamination has become a global phenomenon. Soil in many parts of the world has been contaminated by chemicals and heavy metals due to industrial and agricultural activities, and unregulated waste disposal. In the Europe alone, 2.5 million sites were identified as potentially contaminated with an estimated 342000 sites actually contaminated [1]. A large proportion of chemical-contaminated soil is attributed to environmental release of petroleum products particularly crude oil [2]. Global intensification of oil and gas activities comprising exploration, drilling, production, onshore storage and transportation of petroleum have increased the risk of spillage and leakage of crude oil into the environment [3].

Crude oil is a complex mixture of hydrocarbon and organic compounds, some of which, such as benzene and polyaromatic hydrocarbons are known to pose environmental and health hazards [4]. Soil contaminated by crude oil could render it unsafe for habitation and agricultural activities due to potential human exposure to the harmful compounds of crude oil and bio-accumulation of the compounds in agricultural products [5].

Conventional method of remediating soil contaminated by crude oil involves excavation of the soil followed by subsequent chemical or physical treatments [6]. Chemical treatment utilizes strong oxidants which alter soil properties and are often pH-dependent. Physical treatment applies heat which converts crude oil-related contaminants to simpler compounds but the compounds could still be

harmful [7]. Gaseous compounds from heat treatment may require further treatment prior to environmental release. In addition to the effect on soil properties, health and environment, in-situ excavation of soil is also cost and labour intensive [6].

Besides, in-situ treatment of soil using techniques such as in-situ extraction, soil vapour extraction, air sparging and stabilization can be employed. However, these methods require extensive site characterization to be effective, are complicated and could be costly [7].

Phytoremediation provides a cost-effective alternative to soil remediation by expediting removal of contaminants from soil via physiological processes of plant and microbial activities at the roots of plant [8]. The use of local plant species in phytoremediation is preferred as local plant species adapt well to local climate and soil conditions, thus, having higher probability of success in growing and propagating on contaminated soil [9]. In view of this, the studies of phytoremediation frequently focus on plant species that are common to the areas of study. This study, therefore, aims to examine the potential of plant species common in Malaysia to remediate soil contaminated by crude oil.

2. Literature review

The application of phytoremediation can be traced to the early 1990s during which it was primarily used to remediate soil contaminated with low-risk hazardous substances with potential threat to public health [10]. Research of phytoremediation flourished concurrently and focused on agricultural chemicals [11], heavy metals [12], trinitrotoluene (TNT), petrochemicals and volatile organic compounds [13].

Prior to this, research had already begun in the late 1980s upon the discovery that establishing vegetation could reduce levels of herbicides and excess nutrients [14]. It was also found that deepplanting of hybrid poplar trees enabled remediation of soil contaminated with herbicides and salts beyond the surface level. Subsequently, plant species capable of hyper-accumulating heavy metals in soil were identified via excessively high levels of heavy metals in tissues of those plants [8]. In the 1990s, the research of phytoremediation revolved around field applications which showed encouraging outcomes of contaminants' reduction [15]. However, the early research was carried out in controlled environment and information on the practicability of phytoremediation in treating actual contaminated site was limited. Actual application of phytoremediation was often constrained by the concerns for public health and safety and time factor.

Alongside the identification of the diverse soil-remediating abilities of different plant species, the mechanisms of phytoremediation also raised much research interest. Seven mechanisms have been identified. The first is phyto-extraction also known as phyto-accumulation, wherein contaminants are absorbed by plant roots and translocated to other parts of the plant [16]. Phyto-extraction can be achieved via extraction of high concentrations of contaminants from the soil as for the hyperaccumulators [16], or the uptake of lower concentrations of pollutants while sustaining high growth rate as for the *Populas* sp [17].

Secondly, phytostabilization reduces leaching of contaminants from soil by binding the contaminants to the roots of plants, thus immobilizing them [18]. The roots could also secrete substances that convert harmful heavy metals to less toxic forms [19]. Thirdly, phytodegradation depends on microorganisms attached to and enzymes secreted by the roots to break down contaminants which are then removed via uptake and transpiration. Phytodegradation is well-suited for removal of herbicides, methyl tert-butyl ether and tricholoethylene [10].

Phytostimulation, on the other hand, involves stimulation of soil microbial activity at the rhizosphere for the breakdown of organic contaminants and is effective for degradation of petroleum hydrocarbons and polyaromatic hydrocarbons [10]. Phytovolatilization removes contaminants from soil by volatilizing them into the air and this is often through transforming the contaminants into a less toxic and more volatile form [20]. Rhizofiltration removes contaminants from contaminated waterbodies via the action of roots, thus filtering the water. Its mechanism is similar to phytoextraction but it is used primarily to treat contaminated aquatic environment [21]. Phytodesalination, however, reduces salt concentration of soil using halophytes to improve soil fertility [19].

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The studies of phytoremediation is often localized, focusing on regional plant species capable of treating particular contaminants. Chekol et al. examined the potential of alfalfa, flatpea, sericea lespedeza, deertongue, reed canarygrass, switchgrass and tall fescue commonly encountered in the North America for the treatment of soil contaminated with polychlorinated biphenyl (PCB) and found that the plants significantly reduced PCB in the vegetated pots to various extents, compared with unplanted control [22]. In a similar regional study, Huang et al. revealed the plant growth promoting rhizobacteria associated with tall fescue, Kentucky blue grass and wild rye enhanced phytoremediation [23]. Anh et al. identified two plant species common in Vietnam as arsenic hyperaccumulators and four grasses with potential for treating soil contaminated with lead and zinc [9].

Other studies of phytoremediation narrow down on plant species with reported ability to phytoremediate. Srivastava et al. investigated ferns of the *Pteris* species to hyperaccumulate arsenic since few other *Pteris* ferns have been reported to hyperaccumulate arsenic. They identified three new *Pteris* ferns and reconfirmed *Pteris cretica* as arsenic hyperaccumulators [24]. Phytoremediation-related research is also segmented based on contaminants, with most studies focusing on heavy metals and to lesser extent, petroleum hydrocarbons. Lately, Liao et al. proposed surfactant-enhanced phytoremediation for treatment of crude oil-contaminated soil using maize and reported the ability of rhamnolipid and soybean lecithin to enhance soil microbial activity, hence phytoremediation of crude oil-contaminated soil [2]. Plants with potential for remediation of crude oil-contaminated soil have been identified for instance, *Bassia scoparia* [5], *Salicornia persica* [4] and castor bean [25], and the list is expanding. This study therefore, aims to contribute to the list of plants with potential for treating soil contaminated with crude oil, particularly plants common in Malaysia.

3. Methods

3.1. Soil preparation

Five pots of soil contaminated with 5% crude oil were prepared by mixing the soil uniformly with crude oil. Each pot contained 4kg of soil mixed with 0.2kg of crude oil. The soil was commercially available compost soil for gardening while the crude oil was Miri medium sweet crude (API = 32.30; sulfur content: 0.08%).

3.2. Plant selection and cultivation

Four plant species common in the Miri region of Malaysia, i.e. *Pteris vittata, Epipremnum aureum, Mucuna bracteata* and *Imperata cylindrica*, were chosen for the study because they were readily available and locally widespread while being easy and inexpensive to cultivate. The plants were identified via a review of their potential to remove contaminants from soil and air. Also, the plants have been observed to proliferate in the vicinity of petrol stations and crude oil storage facilities, and their ability to phytoremediate crude oil has not been characterized. The plants were screened for uniformity of fresh weight before planting. The fresh weight of the plants was approximately 0.5kg. Healthy-looking plants with profuse roots were selected to ensure higher success of cultivation in the crude oil-contaminated soil. The roots were trimmed to reduce variability of roots' abundance among the plants. The plants were planted directly in potted soil. One pot served as the control. All the pots were watered twice daily by spraying to maintain sufficient moisture of the soil. The pots were placed in area shaded from rain but with access to sunlight.

3.3. Soil analysis

Soil sampling was conducted on the first week after planting and weekly subsequently for another 5 weeks during which soil samples were collected from each pot at a fixed radius from the plant. The samples collected were sieved with 2mm mesh to separate organic materials and particulate matters [23]. 16 g of soil was collected after sieving for subsequent analysis. The soil was tested for the moisture content, the pH and the crude oil concentration. Soil moisture content was tested to maintain a sufficient level of soil moisture for phytoremediation. pH was tested as plants were known to alter the pH of surrounding soil as phytoremediation occurred [5, 8, 9].

To test for the soil moisture, 10g of soil was placed in a pre-weighed petri dish, which was later placed in an oven and left to dry at a temperature of 105°C until a constant weight was obtained. The percent soil moisture content (MC%) was then calculated with (1) as below [3]:

$$MC\% = \frac{W_2 - W_3}{W_3 - W_1} \times 100\% \quad (1)$$

Where W1 = weight of petri dish (g), W2 = weight of moist soil + petri dish (g) and W3 = weight of dry soil + petri dish (g)

Soil pH was analysed with slurry method by mixing 5g g soil with 5ml of distilled water (1:1 soil to water ratio). The mixture was stirred and left to settle for 30 minutes [3]. A pH meter was then placed in the slurry to measure the soil pH.

The crude oil concentration, as Total Oil and Grease (TOG), was determined with UV-Vis Spectrophotometer. The TOG in 1g of soil was extracted with 10ml N-hexane by sonification and the mixture was separated in a separatory funnel. Extraction of petroleum hydrocarbon with N-hexane is an established method. Petroleum hydrocarbon is largely non-polar and can be effectively extracted with N-hexane [5]. The extract collected was subsequently transferred to a volumetric flask and reconstituted with N-hexane to a final volume of 10ml for analysis with UV-Vis Spectrophometer. The reconstituted further dilution of the crude oil extracts to fit the calibration curve constructed (see figure 1). To measure absorbance, hence concentrations of crude oil, the wavelength of UV-Vis Spectrophometer was set to 360nm [5].



Figure 1. Calibration curve of absorbance against crude oil concentration

4. Results

The control and vegetated pots are denoted with A to E, with A representing unvegetated control, B representing potted soil planted with *Pteris vittata*, C denoting potted soil planted with *Epipremnum aureum*, D denoting potted soil planted with *Mucuna bracteata* and E denoting potted soil planted with *Imperata cylindrica*.

Table 1 shows the concentration of crude oil of contaminated soil in each pot. All potted soil samples showed decreasing crude oil concentration over the span of 6 weeks with C recording the highest percent crude oil reduction, followed by E, B, D and A in descending order. All vegetated pots demonstrated higher percent crude oil reduction than control pot (see table 1).

The rate of crude oil removal for each pot is shown by the gradient of the plots of concentration against time (see figures 2 to 6). All vegetated soil had higher rates of crude oil removal (figures 3 to 6) than that of the control (figure 2) with C (figure 4) exhibiting the highest rate. D came in second (figure 5), E third (figure 6) and B fourth (figure 3) in the rate of crude oil removal. Comparison of the crude oil removal rates in all the pots are also shown in figure 7. D only recorded marginally higher crude oil removal rate than E (figures 5 and 6). Figure 7 shows crude oil removal in the control and experimental pots.

Table 1. Concentration of Crude Oil in Potted Soil

Weeks	Days	Crude Oil Concentration (mg/g)						
		А	В	С	D	Ε		
1	7	38.73	35.41	36.12	32.65	32.37		
2	14	33.75	28.76	31.73	34.49	27.70		
3	21	30.00	29.57	35.32	31.09	27.63		
4	28	29.21	26.19	27.90	28.92	24.29		
5	35	29.81	25.97	25.77	25.19	23.35		
6	42	27.63	22.68	17.93	22.55	19.59		
% overa oil reduct	all crude	28.66	35.96	50.36	30.91	39.47		



Figure 2. Crude oil concentration of A (Control)







Figure 3. Crude oil concentration of B (*Pteris vittata*) compared with A (Control)







Figure 6. Crude oil concentration of E (*Imperata cylindrica*) compared with A (Control)



Figure 7. Comparison of crude oil removal for all pots

The soil pH fluctuated (table 2) though showing an overall decreasing trend with most notable decrease recorded in Week 6 (figure 8). Nonetheless, it cannot be concluded that the plants had decreased the soil pH in the course of phytoremediation as multiple factors could affect the pH for instance, water content and distribution in the soil, and mineral content [8].

Weeks	Days			pН		
		А	В	С	D	Ε
1	7	9.25	9.27	9.25	9.30	9.21
2	14	9.13	9.18	9.22	9.16	9.10
3	21	9.18	9.02	9.05	9.25	9.12
4	28	9.44	9.21	9.12	9.28	9.16
5	35	9.27	9.22	9.08	9.31	9.11
6	42	9.15	8.61	8.81	8.72	8.93

Table 2. pH of potted soil



Figure 8. pH of soil in all pots

Soil moisture content demonstrated fluctuating trends throughout the experimental period and monitoring soil moisture aimed primarily to maintain the moisture at an appropriate level for healthy growth of the plants. The soil moisture ranges from 3% to 10% (see table 3 and figure 9).

Weeks	Days	Soil Moisture (%)					
		А	В	С	D	Ε	
1	7	8.35	9.47	9.45	8.41	7.59	
2	14	4.31	3.21	4.15	3.81	4.25	
3	21	7.52	5.04	5.07	5.83	4.65	
4	28	7.65	5.53	6.09	4.91	5.62	
5	35	6.57	5.89	4.76	5.19	6.26	
6	42	5.92	7.65	8.13	4.64	5.10	

Table 3. Soil moisture content



Figure 9. Percent Soil Moisture

5. Discussion

In all vegetated pots, the percent crude oil removal and crude oil removal rates are higher than the control. This is consistent with previous findings that phytoremediation enhances crude oil removal from contaminated soil [2, 21]. Crude oil removal occurred in unvegetated soil due to the activities of natural-occurring soil microorganisms but the presence of plants increased soil microbial activities and aided the breakdown or absorption of the contaminants [6]. The roots provide large surface area for anchorage of crude-oil degrading microorganisms as well as for absorption, breakdown and removal of crude oil. It is also likely that plant root exudates promote the growth of soil microorganisms as they contain nutrients and energy sources [26].

Pteris vittata has been previously reported as a hyperaccumulator of Arsenic [9, 12, 16] but its effect on crude oil phytoremediation has not been characterized. This study shows *Pteris vittata* as potentially useful for phytoremediation of crude oil contaminated soil. *Epipremnum aureum* is a popular indoor plant with ability to remove indoor pollutants such as formaldehyde [27] and benzene [28]. According to Orwell et al., microorganisms in the rhizosphere were largely responsible for removal of gaseous benzene [28]. *Epipremnum aureum* was also found to show rhizofiltration potential for Co-60 and Cs-137 with transport index of 13.8 for Co and 35.6 for Cs respectively [29]. This study reveals that *Epipremnum aureum* is effective for removal of crude oil from contaminated soil, in addition to the removal of gaseous indoor pollutants and radionuclide reported.

Study on the phytoremediation potential of *Mucuna bracteata* is limited to its ability in accumulating Cd and Pb under soil-leachate conditions to a certain level of soil-leachate [30]. This study highlights that *Mucuna bracteata* can phytoremediate crude oil-contaminated soil. *Imperata cylindrica* has been shown to tolerate Pb-contaminated soil and could remove Pb in soil [31]. Its use for crude oil phytoremediation is underexplored. This study points to such ability of *Imperata cylindrica*.

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All the plants tested in this study exhibited the potential of crude oil phytoremediation with *Epipremnum aureum* topping the list. Observation of plant growth highlighted the high growth rate and adaptability of *Mucuna bracteata* though its rate of crude oil removal only ranked second and its percentage of crude oil removal ranked fourth. This pointed to the long term use of *Mucuna bracteata* in phytoremediating crude oil-contaminated soil and its fast growth could compensate the comparatively lower amount of crude oil removal.

Soil pH is affected by a number of factors, e.g. mineral content and soil texture [3]. The soil used in this study was alkaline and fluctuated within narrow range throughout the study period. Crude oil pollution has been implied to increase acidity of soil, rendering it more toxic [3]. Decreasing pH has been identified as a strategy to enhance phytoextraction of heavy metals [32]. Naturally, the uptake of ammonium by plant has been reported to decrease the pH of rhizosphere due to proton exchange [33]. The moisture contents of soil fluctuated over a larger range during the course of the study owing to factors such as wind, watering frequency, humidity and transpiration rate of plants [5].

The study is limited by duration but it enables the ability of the chosen plants to phytoremediate in the short term to be characterized. This could be important in instances where fast phytoremediation is preferred due to the associated risk of exposure to contaminants. Mixing of crude oil and watering posed constraints on the experiment. Though mixing was extensively conducted, it could not be guaranteed that the crude oil had uniformly mixed with the soil and this could yield disparity in the crude oil concentrations of soil samples taken. Watering, though carefully done, could alter the distribution of crude oil in the potted soil. While plants could naturally change the pH of soil during phytoremediation, the effect of altering pH on phytoremediation of crude oil-contaminated soil was not examined in this study.

6. Conclusion

This study shows that the presence of plants enhances the removal of crude oil from contaminated soil. It unveils the ability of four inexpensive, readily available local plants with significant potential for crude oil phytoremediation with *Epipremnum aureum* being most effective. This study, therefore, contributes to characterization of common local plant species in the phytoremediation of crude oil contaminated soil. Further study can investigate the tolerance of the plant species to different crude oil concentrations for better selection of plant based on the level of contamination.

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