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ARTICLE

Phytoremediation of Soil Contaminated with Crude Oil Using *Mucuna Bracteata*

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ABSTRACT

This study examines the ability of *Mucuna bracteata* DC. to remediate soil contaminated with increasing levels of crude oil up to 20%. It also investigates the effect of fertilizer application on crude oil degradation. Changes in crude oil concentrations, pH and moisture of the soil in eight experimental pots were tracked over a period of 9 weeks. The crude oil levels in soil were analysed as Total Petroleum Hydrocarbons (TPHs) using the UV-Vis spectrophotometer. The study revealed the capacity of *Mucuna bracteata* to phytoremediate soil contaminated with crude oil in all experimental pots though the plant died at 20% contamination towards the end of the experiment. The plant survived up to 15% contamination with that in the fertilized pot showing better physiological conditions. In all instances, fertilized pots showed higher rates of crude oil reduction. The amounts of crude oil degraded in fertilized pots were also higher except at 20% contamination. The soil pH varied over a narrow range throughout the experimental period. Moisture of soil contaminated with 15% and 20% crude oil was higher than that contaminated with 5% and 10% crude oil. *Mucuna bracteata* showed signs of phytoextraction which can be subject to further study. This study contributed a new candidate of phytoremediation for soil contaminated with high level of crude oil.

1. Introduction

Rapid development over the recent decades has led to increased anthropogenic activities such as industrialization, transportation, agriculture and urbanization. These activities have left behind a host of pollutants, some are short-lived while others have long-lasting environmental impacts [1, 2]. Many of the pollutants are petrochemicals originated from crude oil which is the driving force behind a multitude of anthropogenic activities. Increased industrialization, agriculture, transportation and urbanization spurred the demand for petrochemicals, hence crude oil exploration and production [3]. This is evident in Figure 1 below showing an overall increasing trend of crude oil production since 1960.

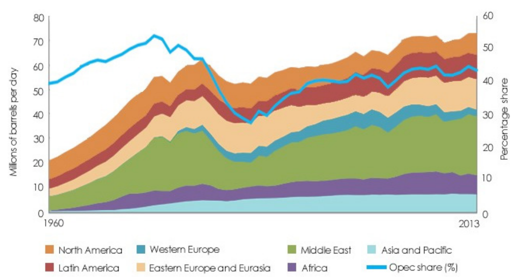

Figure 1. Trend of World Crude Oil Production [4]

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With approximately 70% of worldwide oil production coming from onshore drilling, the drilling sites and their surrounding receive substantial amount of drill cuttings, used drilling mud and accidental spillage. Accidental spillage is also possible during transportation and transfer of crude oil and petrochemicals, resulting in potential widespread soil contamination [5]. A study by Wang et al. showed that crude oil altered the physical and chemical properties of soil, resulting in, for instance, increased soil pH and reduced phosphorus level. This could affect soil structure and function [6].

According to BBC, 16.1% of the soil in China and 19.4% of China's arable land were contaminated, mainly with heavy metals [7]. The Food and Agriculture Organization (FAO) of the United Nations revealed 14% of land degradation worldwide [8]. An estimated 350,000 contaminated sites have been identified in Europe and most of them have been contaminated by petrochemicals [9]. In Nigeria, petroleum exploitation has resulted in the entry of crude oil into land, waterbodies and marshes [10]. These petrochemical pollutants can be mobile and persistent in the environment, giving rise to health concerns [1]. Exposure to polycyclic aromatic hydrocarbons (PAHs) present naturally in crude oil or as by-products of incomplete combustion and crude oil degradation have been associated with cancer, mutation and malformation of embryos. PAHs are also moderately or highly toxic to aquatic lives and birds [11].

Soil remediation strategies are commonly categorized into three, i.e. chemical, physical and biological. Physical method involves application of heat, washing agent and air to degrade pollutants while chemical treatment uses oxidizing or reducing agents [12]. Biological treatment makes use of bacteria present in the soil or attached to the roots of plants as well as specific strains of bacteria introduced into the soil to break down the contaminants. Physical and chemical methods are fast but can be intrusive, resulting in changes of soil properties. Biological method though comparatively slower, does not alter the soil properties and produce harmful intermediates as with physical and chemical remediation [12]. Microwave remediation is increasing employed due to its cost-effectiveness at small scale and its ability to remove petroleum hydrocarbons quickly. However, at a larger scale, the remediation becomes costly and the percent organic removal depends on the processing system used [13].

Bioremediation, particularly phytoremediation has been gathering attention because it is inexpensive and uncomplicated in application. Plants have been shown to mineralise heavy metals and decompose petroleum hydrocarbons via their profuse root system. The mechanisms involved in phytoremediation typically comprise phytoextraction, phytodegradation, phytovolatilization, phytostabilization and rhizodegradation [14]. Phytoremediation is an in-situ soil treatment that reduces contaminants as plants grow. The selection of plant species capable of surviving in and remediating soil contaminated with target contaminants is crucial [15]. The study of phytoremediation was initiated as treatment for soil contaminated with heavy metals using *Thlaspi caerulescens* J.Presl & C.Presl and *Viola calaminaria* Lej. [16]. Since then, research has been extended to investigate its ability to remediate other contaminants.

Phytoremediation has been demonstrated to provide promising treatment of soil contaminated with petroleum hydrocarbons, PAHs, and polychlorinated biphenyls (PCBs) [17]. *Brassica juncea* (L.) Czern was shown to degrade total petroleum hydrocarbons (TPHs) though its growth was inhibited on soil with heavy contamination of TPHs [18]. The primary mechanisms for TPHs removal were rhizodegradation and humidification, involving the breakdown of TPHs in the root zone by microbial actions enhanced by root exudates. The enhanced microbial activities around the roots is also called the rhizosphere effect [19]. Macci et al. tested the ability of *Populus alba* L. (Poplar), *Cytisus scoparius* (L.) Link and *Paulownia tomentosa* (Thunb.) Steud in the remediation of soil contaminated with TPHs and PCBs [20]. The study showed significant removal of the organic contaminants with Poplar being the most effective. Comparison has also been made between phytoremediation, bioremediation and a combination of both in the remediation of sandy soil spiked with 2.5%, 5.0% and 10.0% of TPHs. The results revealed that phytoremediation with alfalfa achieved an efficiency of 99.9% in comparison with bioremediation and both combined, having an efficiency of 98.7% and 99.0% respectively [21]. Anyasi and Atagana studied 28 indigenous plant species growing on soil contaminated with hydrocarbon around petroleum pipelines and found that certain plants could tolerate high levels of TPHs. The study highlighted the ability of *Chromolaena odorata* (L.) R.M.King & H.Rob, *Aspilia Africana* (Pers.) C.D.Adams, *Chloris barbata* Sw., *Paspalum vaginatum* Sw., *Bryophyllum pinnatum* (Lam.) Oken, *Paspalum scrobiculatum* L., *Cosmos bipinnatus* Cav., *Eragrostis atrovirens* (Desf.) Trin., *Cyperus rotundus* L. and *Uvaria chamae* P.Beauv to remediate the soil [22].

Globally, there are numerous studies of phytoremediation, often characterized by the selection of plant species common in the areas of study. Such studies have also been conducted in Malaysia with plant species commonly encountered in the country. In line with the findings of Anyasi and Atagana, Sanusi et al. reported the ability of

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Paspalum vaginatium in remediating soil contaminated with hydrocarbon [22, 23]. A similar study was conducted by Idris et al. to identify native species exhibiting the potential of phytoremediating sites contaminated with very high concentration of TPHs which again confirmed the potential of *Paspalum vaginatum* for such purpose [24]. The study revealed a range of other plant species with such capacity, i.e. *Paspalum scrobiculatum* reported previously by Anyasi and Atagana [22], *Eragrostis atrovirens*, *Cayratia trifolia* (L.) Domin, *Chloris barbata*, *Pycreus polystachyos* (Rottb.) Beauv and *Ischaemum timorense* Kunth [24]. In addition, *Jatropha curcas* L. has been shown to remove 56.6% and 67.3% of waste lubricating oil from soil contaminated with 2.5% and 1% of the oil respectively. The removal efficiency was increased to 89.6% and 96.6% by adding organic waste to the contaminated soil [25]. Another phytoremediation study with *Ludwigia octovalvis* (Jacq.) P.H.Raven revealed its ability to remove 79.8% of TPHs at a soil contamination concentration of 2g/kg over 72 days [26].

Mucuna bracteata is a leguminous plant widely found in Malaysia and is a popular cover crop for oil palm plantations. The plant is known to proliferate quickly at a rate of about 10-15 cm/day and shows the ability to grow in different types of soil. Phytoremediation research with *Mucuna bracteata* has been conducted for heavy metals unveiling its ability to accumulate Cd and Pb to a certain extent [27]. However, very few studies of *Mucuna bracteata* are related to its ability in remediating soil contaminated with crude oil. Tang and Juan conducted a screening study of the phytoremediation potential of five plants and found *Mucuna bracteata* removing crude oil to a certain extent [14]. This study augments the study of Tang and Juan [14] by examining the crude oil removal capacity of *Mucuna bracteata* in potted soil spiked with different concentrations of crude oil, with and without fertilization.

2. Methods

2.1 Soil Preparation and Plant Cultivation

The experiment was conducted with commercially sourced loamy soil which is suitable for the growth of *Mucuna bracteata* [27]. The soil was screened to remove materials such as stones and detritus. The screened soil was spiked with crude oil at different concentrations before being transferred to pots. The crude oil was Miri medium sweet crude (API = 32.3^{o}; sulphur content: 0.08%) [14]. Eight pots were prepared as follows, four for phytoremediation without fertilizer and another four for fertilizer-aided phytoremediation.

Pot A: 2kg soil + 5% crude oil (not fertilized)

Pot B: 2kg soil + 10% crude oil (not fertilized)

Pot C: 2kg soil + 15% crude oil (not fertilized)

Pot D: 2kg soil + 20% crude oil (not fertilized)

Pot E: 2kg soil + 5% crude oil (fertilized during phytoremediation)

Pot F: 2kg soil + 10% crude oil (fertilized during phytoremediation)

Pot G: 2kg soil + 15% crude oil (fertilized during phytoremediation)

Pot H: 2kg soil + 20% crude oil (fertilized during phytoremediation)

Mucuna bracteata was then planted in the pots. The plants were grown from seeds sourced commercially which were left to germinate and grow in soil-filled polybags for 6 weeks to ensure they had the same age and size upon transplant to the potted soil. During transplant, the seedlings were 6-week old, characterized by trifoliate leaves with size (leaf length) ranging from 8cm to 10cm. The plants were weighed and trimmed prior to transplant to minimize biomass variability. All the pots were manually watered twice daily by spraying to ensure sufficient moisture for plant growth. The pots were placed in area exposed to sun but protected from rain. 10 ml of liquid fertilizer (with NPK at a ratio of 10-8-7, zinc (1%), magnesium (1%) and micronutrients (concentration not specified), i.e. manganese, boron, copper and molybdenum) was added to the fertilized pots weekly.

Growth and physiological conditions of the plants, particularly the colour and number of leaves were observed throughout the experimental duration of 9 weeks. During the experimental duration, Pots A to H were placed in area receiving sunshine during daytime but shaded from rain to better control the soil moisture. Variations in the concentration of crude oil, pH and moisture in all the pots were tracked weekly throughout the duration. The methods of sampling and analysis are described below. A constraint of the experiment is the amount of soil used in each pot. The reason that the volume of soil has been limited was to enable more even distribution of roots throughout the soil for crude oil phytoremediation. Therefore, the sampling was conducted only once weekly for each pot.

2.2 Soil Analysis

The spiked soil in each pot was sampled on day seven after the transplant and every week subsequently at a fixed radius from the plant for analysis of crude oil content for 9 weeks or 63 days. 20 g of soil sample was collected from each pot. The amount of soil needed for weekly analysis was equivalent to only 1% of the total 2 kg of soil in each pot. A total of 9% of soil was drawn from each pot throughout the 9 weeks and that was an acceptable prac-

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tice based on previous literature [10, 17, 18]. 10g of the soil sample was used for moisture testing while another 10g was left to air dry at room temperature for 8 hours and sieved with 2mm mesh. Sieving separates organic materials and particulate matters as well as allows a narrower range of soil particle sizes to be obtained [10, 14].

To test the crude oil concentration, 1g of air-dried soil was transferred to a separating funnel after which 10ml of N-hexane was added. N-hexane has been commonly used as a solvent for TPHs. The mixture was shaken and left to stand until the soil particle settled. The mixture was passed through a funnel with filter to remove the soil and the filtrate was collected in a 10ml volumetric flask. The filtrate was reconstituted with N-hexane to a final volume of 10ml for analysis with UV-Vis Spectrophotometer. The wavelength for analysis of TPHs is 360nm [14, 17].

Variations of soil moisture and pH were also monitored weekly to maintain the moisture level of soil appropriate for the plant growth and to examine how phytoremediation altered the pH of soil. Crude oil contamination can affect soil pH. Soil pH might therefore change during the course of phytoremediation. ASTM D2974 method was used to test the soil moisture content. 10g of the soil set aside earlier on (wet sample) was transferred to a petri dish. The sample was dried in an oven at 125°C overnight, after which it underwent repeated weighing and drying until a constant weight was obtained [14, 17, 28]. The percentage of soil moisture was then calculated using the following equation.

$$Soil\ moisture\ (\%) = 100 - \left(\frac{Weight\ of\ dry\ soil}{Weight\ of\ wet\ soil} \right) \times 100\% \quad (1)$$

The soil pH was tested by mixing 5g of sieved air-dried soil to water at a ratio of 1:1. The mixture was stirred and left to stand for around 30 minutes. The pH was taken using a pH meter.

3. Results and Discussion

3.1 Observation of Plant's Physiological Condition

The plants in all pots were physiologically well in the

Table 1. Photos Showing physiological Conditions of Plants on Day 1, Day 30 and Day 63 of the Experiment

Day 1	Day 30	Day 63
Plant in Pot A (5% crude oil without fertilizer)		
		
Plant in Pot E (5% crude oil with fertilizer)		
		

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Plant in Pot B (10% crude oil without fertilizer)



Plant in Pot F (10% crude oil with fertilizer)



Plant in Pot C (15% crude oil without fertilizer)



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Plant in Pot G (15% crude oil with fertilizer)

Plant in Pot D (20% crude oil without fertilizer)

Plant in Pot H (20% crude oil with fertilizer)

first week of experiment (Table 1). Plants in fertilized soil generally showed increased leaves. The plants in Pots A and E showed healthy growth throughout the experimental period though turning slightly yellowish around day 30 most likely attributed to weather condition (Table 1). Plants in Pots B and F also demonstrated satisfactory health with the leaves more yellowish compared to those in Pots A and E towards the end of the experiment (Table 1). The leaves of plants in Pots C and G reduced over the experimental period with brown spots developing on the leaves possibly due to accumulation of crude oil (Table 1). On day 63, both unfertilized and fertilized plants exposed to 15% crude oil showed stunted growth with the former more severely stunted. Plants in Pots D and H also depicted reduction of leaves and development of brown spots on leaves (Table 1). The plants died at the end of the experiment.

3.2 Crude Oil Removal

The crude oil in all pots decreased over the experimental duration with higher removal shown in fertilized pots generally, showing that fertilization increases remediation efficiency (Table 2). *Mucuna bracteata* showed the ability to remediate soil contaminated with up to 20% of crude oil though the plant died at the end of the experiment (Tables 1 and 2). This indicates that *Mucuna bracteata* is tolerant to 15% crude oil contamination. The percent crude oil removal decreased with increasing levels of contamination between Week 1 and Week 9 and *Mucuna bracteata* showed a range of crude oil removal between 12mg/g to 22mg/g in the experimental pots (Table 2). When presented as crude oil removal rates indicated by the gradients of the best-fit straight lines in Figures 2 to 5, the rates of fertilized pots increased up to 15% contamination level and decreased subsequently. The rates of crude removal for unfertilized pots peaked at 10% crude oil contamination (Figure 3), implying that fertilization enabled phytoremediation to occur effectively at a higher level of contamination. The rates of crude oil removal are invariably higher in the fertilized pots than the unfertilized pots (Figures 2-5). The highest rate of crude oil removal was reported from fertilized pot contaminated with 15% crude oil (Figure 3).

Table 2. Concentration of Crude Oil in Sampled Soil

Week	Crude Oil Concentration (mg/g)							
	5%		10%		15%		20%	
	F[a]	NF[a]	F	NF	F	NF	F	NF
1	38	34	51	50	68	71	88	84
2	36	33	47	48	65	65	84	79
3	35	33	44	45	61	60	84	79
4	35	32	43	45	61	62	83	77
5	34	31	43	45	57	61	80	75
6	34	31	42	44	57	64	78	76
7	31	29	40	41	56	62	77	74
8	28	28	38	38	52	57	75	73
9	24	25	37	37	46	55	76	72
Week 1 – Week 9	14	9	14	13	22	16	12	12
% Removal[b]	36.8	26.5	27.5	26	32.4	22.5	13.6	14.3

Note: [a] F = fertilized; NF = not fertilized

[b] % Removal (from Week 1 to Week 9) =

$$\frac{\text{Crude oil concentration in Week 1} - \text{Crude oil concentration in Week 9}}{\text{Crude oil concentration in Week 1}} \times 100$$

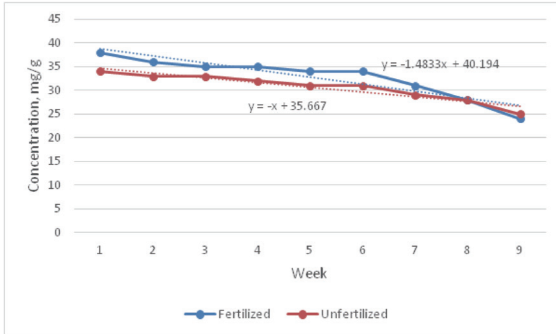

Figure 2. Crude Oil Concentration by Week at 5% Contamination

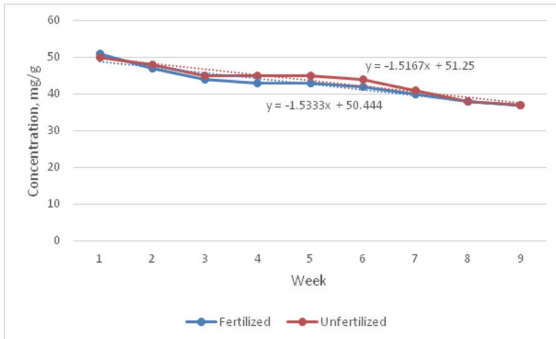

Figure 3. Crude Oil Concentration by Week at 10% Contamination

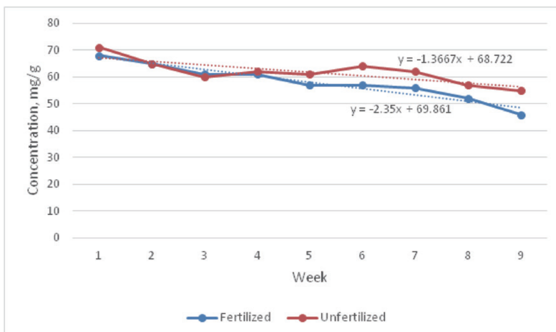

Figure 4. Crude Oil Concentration by Week at 15% Contamination

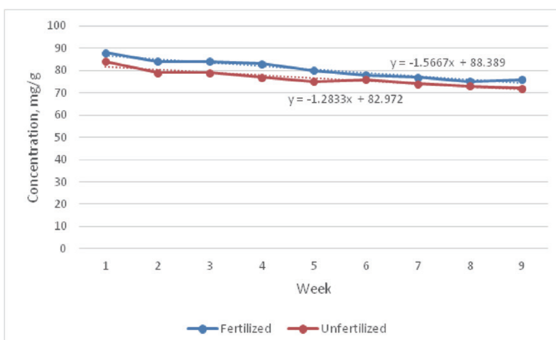

Figure 5. Crude Oil Concentration by Week at 20% Contamination

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Fertilizer has been shown in multiple studies to enhance phytoremediation. Lin and Mendelssohn found that application of fertilizer increased the rate of oil degradation in soil spiked with crude oil and highlighted the potential of fertilization to augment phytoremediation of wetlands contaminated with crude oil [29]. Unlike this study, Lin and Mendelssohn examined the phytoremediation capacity of *Spartina alterniflora* and *Spartina patens*, two species commonly found in marshlands [29]. The study also showed both plants have limits to their tolerance for crude oil beyond which they were killed. It was proposed in the study that fertilizer increased microbial activities in soil and plant biomass, resulting in higher crude oil removal [29]. In line with Lin and Mendelsson, Dominguez-Rosado and Pichtel reported increased crude oil removal from contaminated soil with application of NPK fertilizer and phytodegradation was the mechanism behind effective crude oil degradation by clover and sunflower/mustard treatments [30]. In addition, Olsen et al. recommended inclusion of fertilization and clipping of aboveground biomass in phytoremediation strategies as they enhanced plants proliferation, hence removal of PAHs [31]. A more recent study showed fertilizer application significantly contributed to the efficiency of phytoremediation [32]. *Pinus densiflora* used for phytoremediation increased microbial activities and its growth did not appear to be adversely affected by petroleum contamination at 6000mg/kg unlike other plants tested, i.e. *Populus tomentiglandulosa* and *Thuja orientalis* [32]. Nonetheless, the contamination level is much lower than those in this experiment. This highlights the potential of *Mucuna bracteata* to treat soil contaminated with a higher level of crude oil up to 10% either fertilized or unfertilized, without comprising its growth. At 15% contamination, though the plant's growth was compromised, phytoremediation continued to occur especially in the fertilized pot (Tables 1 and 2).

While most phytoremediation studies showed phytodegradation, especially rhizodegradation as the mechanism underlying the removal of petroleum hydrocarbon from contaminated soil [10, 14, 22, 31], this study suggested the ability of *Mucuna bracteata* to phytoextract crude oil. This was deduced from the formation of dark spots on the leaves of the plant at 15% and 20% contamination. Further study can examine the compositions of the dark spots and whether the compositions could be further metabolized by *Mucuna bracteata*.

3.3 Soil pH and Moisture

Soil pH in the fertilized pots fluctuated over a narrow range of 6 to 7.5 (Figure 6) while that in the unfertilized pots fluctuated over a wider range of 6 to 8 (Figure 7). In both types of pots, the initial and final soil pH decreased with increasing levels of crude oil contamination demonstrating that increasing crude oil tends to lower soil pH. However, Njoku et al. observed positive correlation between crude oil level and soil pH and suggested that increasing crude oil level in soil leads to increased soil pH [17]. Degradation of crude oil in the course of phytoremediation was deemed to produce organic acid which lowered the pH [33].

In this experiment, the soil pH fluctuated over a narrow range and a significant positive correlation between crude oil concentration and pH cannot be established except for the observation of the trendlines in Figures 6 and 7 showing more highly contaminated soil generally has slightly lower pH. This is in fact, parallel to the findings of Ogbogodo et al. that high crude oil level in soil did not affect the soil pH significantly [28]. The decrease in pH reported by Njoki et al. with advancing phytoremediation was masked by the fluctuating pH readings [17].

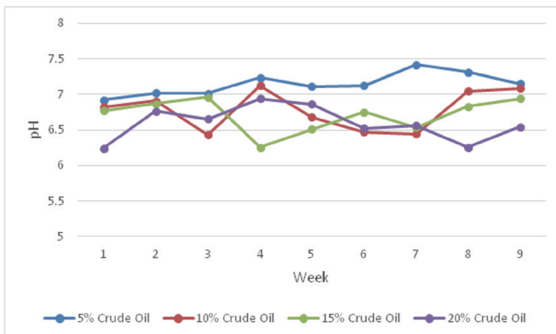

Figure 6. Soil pH of Fertilized Pots at Different Contamination Levels

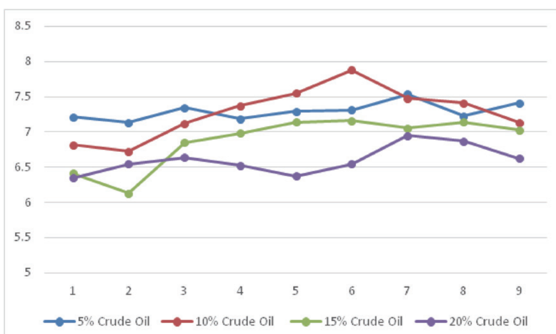

Figure 7. Soil pH of Unfertilized Pots at Different Contamination Levels

The soil moisture data formed two obvious clusters where soil contaminated with 5% and 10% crude oil recorded lower moisture than soil contaminated with 15% and 20% crude oil (Figures 8 and 9). This may imply that crude oil inhibits moisture loss from soil by forming an impervious layer on soil particles. By the same token, crude oil could also make soil moisture less available to phytoremediating plants, thus inhibiting plant growth. This assumption is supported by Andrade et al. that crude oil decreased permeability and infiltration of water into the soil, resulting in retention of water on soil surface and a drier subsurface layer [34]. Toxicity of high crude oil concentration and drier subsurface soil inhibit root growth, hence reduced capacity of the plant for transpiration. This also contributes to higher moisture in soil contaminated with crude oil to a higher level. The significant differences between the soil moisture contents of fertilized (Figure 8) and unfertilized (Figure 9) pots cannot be established in this study.

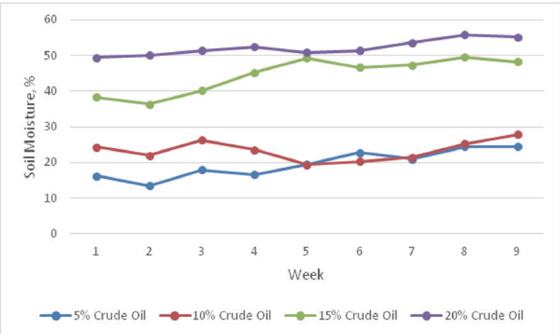

Figure 8. Soil Moisture of Fertilized Pots at Different Contamination Levels

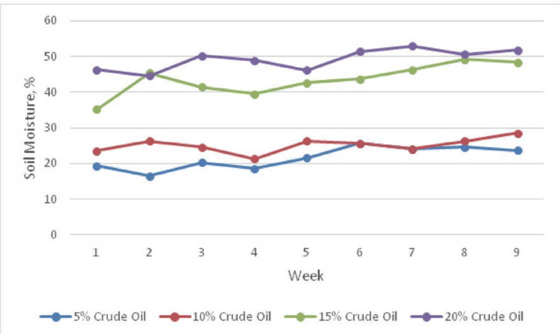

Figure 9. Soil Moisture of Unfertilized Pots at Different Contamination Levels

Phytoremediation studies are inherently subject to multiple variables. The mixing of soil with crude oil, albeit carefully carried out, could not guarantee a homogeneous mixture with crude oil evenly distributed throughout the soil. This was a major contributor of the fluctuation in crude oil concentrations in some instances. Crude oil distribution in soil could also be affected by factors such as watering, soil particle sizes and root distribution affecting the extent of phytoremediation since rhizodegradation is the main means of crude oil removal. Time constraint imposed on this study was also an important drawback as the endpoints of phytoremediation could not be determined without sufficient time given for the crude oil concentrations in the experimental pots to plateau.

4. Conclusion

This study shows the ability of *Mucuna bracteata* to survive in and phytoremediate soil contaminated with crude oil up to 15%. It demonstrates that *Mucuna bracteata* is a potential candidate for phytoremediation of relatively heavy crude oil contamination and fertilizer enhances crude oil removal. The high growth rate of *Mucuna bracteata* further enhances its ability to phytoremediate. This study also unveils potential capacity of *Mucuna bracteata* to phytoextract in addition to rhizodegrade like other crude-oil degrading plants. Phytoextraction capacity of *Mucuna bracteata* could therefore be subject to more extensive research.

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