The Effect of Fertilizer on *Epipremnum Aureum* in Phytoremediating Soil Contaminated with Crude Oil

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Abstract. *Epipremnum aureum* is a common ornamental foliage which is used mainly for indoor decoration. It demonstrates tolerance to low light environment and the ability to remove atmospheric chemicals such as gasoline and formaldehyde. A previous screening study showed the ability of *Epipremnum aureum* to remove crude oil from soil. Further to the screening study, this study examined the effect of fertilization on phytoremediation of crude oil-contaminated soil by *Epipremnum aureum Epipremnum aureum* was grown in pots with soil contaminated with 5%, 10% and 15% of crude oil by weight respectively, with and without the addition of fertilizer. In line with previous study, *Epipremnum aureum* showed the ability to remove crude oil contaminants. The plant demonstrated highest crude oil removal rate in pots with 15% contamination. Application of fertilizer enhanced the removal of crude oil in all experimental pots by Week 6, hence increasing the rate of crude oil decline over the experimental duration. It is deemed that application of fertilizer provides nutrients to promote plant growth and proliferation of roots which enhance rhizodegradation of crude oil in soil. This study extends on the existing knowledge that *Epipremnum aureum* can phytoremediate crude-oil contaminated soil by characterizing its tolerance to crude oil and its response to fertilization, which is crucial to its practical application in phytoremediation. Phytoremediation presents a promising yet cost-effective measure in treating contaminated land. Further study can examine the optimum fertilizer concentration for phytoremediation and the tolerance level of *Epipremnum aureum* to crude oil contamination.

1. Introduction

Intensifying anthropogenic activities ensuing population rise and the increase of industrial and agricultural activities have resulted in widespread pollution of the water, air and land. Land pollution is often manifested as soil contamination due to the presence of xenobiotic chemicals [1]. These chemicals frequently comprise petroleum hydrocarbons, polynuclear aromatic hydrocarbons, pesticides and heavy metals [2]. The detection of microplastics in terrestrial ecosystems such as soil also suggest contamination of soil by these fine plastic fragments which can be hard to remove [3]. Contamination of soil with petroleum hydrocarbon has been a persistent environmental issue the global communities are still combating. Activities such as onshore drilling, refining and storage of crude oil leave behind significant amount of petroleum hydrocarbons. Accidental spillage and leakage of petroleum products during transportation also causes entry of petroleum hydrocarbons into the terrestrial ecosystems in addition to improper disposal of petroleum products [2, 4]. It was estimated that the Europe alone has 2.5 million potentially contaminated sites of which approximately 342000 sites have been declared
contaminated. The contamination was attributed mainly to heavy metals and petroleum products, indicating that petroleum products are one of the major soil contaminants [5].

Soil contamination by crude oil and petroleum products has a number of implications. Crude oil can alter the physical properties of soil by adsorbing on soil surfaces, thus, affecting porosity and permeability of soil [6]. Carbon-rich and nitrogen-deficient crude oil can also alter organic contents, pH, conductivity as well as carbon to nitrogen and carbon to phosphorus ratios of soil, which potentially affect the soil suitability for agriculture [7]. Nickel and vanadium commonly found in petroleum products can result in eco-toxicity following their release with petroleum products into soil [8]. Besides, crude oil contamination can result in modification of microbial population and enzyme system in soil [4]. Soil contaminated with crude oil has been shown to have lower germination rate, resistance to pests [9] and fertility due to decreased availability of nitrogen and phosphorus for the growth of crops [10]. Crude oil consists of a mixture of aliphatic, aromatic and organometallic constituents, many of which are harmful to human health, particularly the polycyclic aromatic hydrocarbons. These compounds may exhibit carcinogenic, mutagenic and teratogenic effects as they find their ways to the bodies of human and animals [11].

With widespread contamination of soil by petroleum products, removal of these pollutants has attracted enormous research interest. Four major removal methods of these pollutants have been identified, i.e. physical remediation, chemical remediation, microbiological remediation and phytoremediation [1]. Physical remediation involves soil removal and replacement as well as treatment of soil with heat and microwave which can be energy and cost intensive [1]. Chemical remediation uses elution, solvent extraction and oxidation which may leave behind an array of chemicals harmful to the environment [12]. Microbiological remediation uses microorganisms to degrade petroleum products in soil and the activities of microorganisms often depend on the soil conditions as well as the presence of nutrients [13]. Microbiological remediation, phytoremediation and a mixture of both are collectively known as bioremediation. Bioremediation provides a cost-effective alternative to removal of petroleum products from soil. As microbiological remediation is subject to numerous variables that affect the performance of remediating bacteria, phytoremediation has gradually gained popularity [7].

Phytoremediation works based on the interactions between plants, microorganisms and environment. It removes contaminants from soil via simulating microbial activity in the root zone as the roots secrete nutrient-rich exudates which also may contain contaminants degrading enzymes, or via direct absorption and transformation of contaminants [9]. For crude oil removal, studies have suggested degradation or transformation by microorganisms at the root zone as the main mechanism [14]. Many studies have been conducted for phytoremediation of crude oil contamination either globally or locally. In the North American, plants such as flatpea, alfalfa, reed canarygrass and deertongue, among others, have been shown to reduce polychlorinated biphenyl [15]. Bassia scoparia [14], Salicornia persica [16] and castor bean [17] have been reported as potential candidates for remediation of soil contaminated with crude oil. Locally, Tang and Angela showed the ability of Pteris vittata, Epipremnum aureum, Mucuna bracteata and Imperata cylindrica to remove crude oil from soil [12]. This was the first study that demonstrated the phytoremediating ability of Epipremnum aureum and Mucuna bracteata for crude oil.

Subsequently, a study was conducted by Tang and Law [18] to further investigate the ability of Mucuna bracteata in phytoremediating soil contaminated with crude oil up to 20% with and without application of fertilizer. The study demonstrated survival of Mucuna bracteata up to 15% crude oil contamination and better removal of crude oil in all pots where fertilizer was applied. This was in line with other studies pointing to the positive effect of fertilizer on phytoremediation [19, 20, 21]. However, no further studies of similar nature have been conducted for Epipremnum aureum which is a hardy, low-cost and widely available ornamental plant. This study therefore aims to examine the tolerance of Epipremnum aureum
to different levels of crude oil contamination and the effect of fertilizer application on its ability to phytoremediate.

2. Methods

2.1. Soil preparation and plant cultivation

Loamy soil for cultivation of *Epipremnum aureum* was purchased and sieved through 50mm mesh to remove large organic debris and rock fragments. The soil was separated into parts of 2kg for both the control and experimental pots. Fertilizers were not applied to the plants in control pots while for experimental pots, the plants were fertilized.

Soil for control and experimental pots was mixed with crude oil at concentrations of 5%, 10% and 15% respectively by weight, as uniformly as was practicable. Crude oil used for the experiment was Miri medium sweet crude with 0.08% sulphur and an API of 32.3°. The fertilizer used was liquid NPK at a ratio of 10-8-7 fortified with 1% zinc, 1% magnesium and micronutrients of unspecified concentrations.

Six control and experimental pots were prepared as follows:

- **Pot A**: 2kg soil + 5% crude oil (without fertilizer application)
- **Pot A1**: 2kg soil + 5% crude oil (with fertilizer application)
- **Pot B**: 2kg soil + 10% crude oil (without fertilizer application)
- **Pot B1**: 2kg soil + 10% crude oil (with fertilizer application)
- **Pot C**: 2kg soil + 15% crude oil (without fertilizer application)
- **Pot C1**: 2kg soil + 15% crude oil (with fertilizer application)

Healthy foliage of *Epipremnum aureum* weighing approximately 50g each, with comparable size was planted in polybags for 1 week prior to transplant to the pots above. Growth of plants in the pots were observed over the experimental duration of 6 weeks. The pots were placed at area exposed to sufficient sunlight but shaded from rain to minimize redistribution of crude oil due to rain splashes. The pots were watered twice daily by means of sprayer with similar amount of water to ensure sufficient moisture for *Epipremnum*’s survival. The volume of water used during each spraying was approximately 50ml.

20g of soil was collected weekly for analysis of the crude oil and moisture contents, and the pH.

2.2. Soil analysis

20g of soil from each pot was collected for analysis weekly after the transplant for 6 weeks. The soil was divided into two parts of 10g. The first 10g was tested for its moisture content using the ASTM D2974 method by evenly spreading it out on a petri dish and drying it in an oven at 125°C overnight until a constant weight was obtained [18]. The weight of soil before and after drying was recorded and the moisture content calculated with the following formula:

\[
\text{Soil moisture} (\%) = 100 - \left( \frac{\text{Weight of dry soil}}{\text{Weight of wet soil}} \right) \times 100\% \tag{1}
\]

The remaining 10g of soil was air-dried at room temperature for 8 hours and sieved with 2mm mesh. 5g of the sieved soil was mixed with water at a ratio of 1:1, stirred and left for 30 minutes before being tested for pH with a pH meter [22]. The purpose of moisture and pH analyses was primarily to ensure that these parameters were monitored for optimal plant growth. 2g of the sieved air-dried soil was transferred to a separating funnel and mixed with 10ml of hexane. The mixture was agitated to strip the
crude oil from the soil and was left to stand until the soil had completely settled before filtration to obtain the filtrate containing crude oil extract. The filtrate was subsequently reconstituted with hexane to a final volume of 10ml before being transferred to a cuvette bound for UV-Vis Spectrophotometer [12, 18]. The wavelength of the spectrophotometer was set at 360nm [22].

3. Results and discussion

Physiological condition of the plants was observed for 6 weeks. First two weeks after the transplant, the plants in all pots showed signs of yellowing and wilting as they were probably still adapting to the soil in the pots and the crude oil therein. After two weeks, the plants showed improved physiological conditions with greener leaves. Plants on potted soil contaminated with 5% crude oil showed better health compared to those on soil contaminated with 10% and 15% crude oil, wither fertilized or unfertilized. Generally, it can be concluded that *Epipremnum aureum* can survive in soil contaminated with 15% of crude oil. The limit of tolerance has not been identified in this study. Therefore, it is uncertain if *Epipremnum aureum* can survive higher levels of crude oil contamination.

3.1. Crude oil removal

*Epipremnum aureum* exhibits the ability to remove crude oil in all pots and the rate of crude oil removal, as indicated by the gradients of the trend lines, showed a rising trend as the level of crude oil contamination increased (see Figures 1 to 3). Application of fertilizer in all instances increased the rate of crude oil removal with more significant increase seen in Figure 2 and Figure 3. This was parallel to the findings of Tang and Law that fertilizer application increased the rates of crude oil reduction in soil planted with *Mucuna bracteata* [18]. Similar findings were reported by Kai et al. that soil amendment with perlite and higher basal fertilizer rates increased the removal rates of total petroleum hydrocarbon by *Zinnia hybrid* in soil contaminated with crude oil [19]. It is deemed that application of fertilizer provides nutrients to promote plant growth and proliferation of roots which enhance rhizodegradation of crude oil in soil [20].

![Figure 1: Weekly concentration of crude oil in soil contaminated with 5% crude oil](image-url)
Percent removal of crude oil at Week 6 was calculated by expressing the difference between the crude oil concentration in Week 6 and that in Week 1, in relation to the crude oil concentration in Week 1 as percentage. The percent of crude oil removed was the highest in potted soil contaminated with 5% of crude oil where fertilizer was applied. Percent of crude oil removed was generally observed to be higher in pots with fertilizer applied than those without fertilizer application (Figure 4). In comparison to *Mucuna bracteata* [18], *Epipremnum aureum* showed significantly higher removal of crude oil in fertilized pots and unfertilized pots (a difference of approximately 29% and 14% respectively) with soil contaminated with 5% crude oil over 6 weeks. At 10% and 15% contamination, the percent crude oil
removed by *Epipremnum aureum* was also higher than *Mucuna bracteata* by the range of 3% to 8% [18]. This agreed with the screening study of local plants for crude oil phytoremediation by Tang and Angela which revealed that *Epipremnum aureum* performed better than *Mucuna bracteata* in terms of the amount of crude oil removed over 6 weeks at 5% contamination [12].

![Figure 4: Percent crude oil removed from potted soil at different levels of contamination](image)

3.2. Soil pH

The soil pH of all pots fluctuated within a narrow range of 5.8 to 6.35 (Table 1), indicating that there was no significant variation of the pH within the experimental duration and variability of phytoremediation due to the influence of pH was minimized. The pH of soil used in this study is weakly acidic. Soil pH influences nutrient availability and toxicity [23]. Commercially sourced soil commonly has pH in the range of 5.5 to 7.0 which is suitable for most crops [20].

**Table 1.** pH of soil contaminated with 5%, 10% and 15% crude oil respectively.

| Week | 5% Crude Oil | 10% Crude Oil | 15% Crude Oil |
|------|--------------|---------------|---------------|
|      | Without Fertilizer | With Fertilizer | Without Fertilizer | With Fertilizer | Without Fertilizer | With Fertilizer |
| 1    | 6.04          | 6.02           | 6.23          | 6.13          | 6.31          | 6.22          |
| 2    | 6.02          | 5.81           | 6.27          | 6.17          | 5.79          | 6.25          |
| 3    | 6.24          | 6.07           | 6.13          | 5.99          | 5.98          | 6.35          |
| 4    | 6.24          | 6.01           | 6.20          | 6.13          | 5.99          | 6.28          |
| 5    | 6.19          | 6.08           | 5.97          | 6.08          | 6.30          | 6.22          |
| 6    | 6.17          | 6.02           | 5.99          | 6.08          | 6.37          | 6.34          |

3.3. Soil Moisture

The soil moisture is shown Table 2. The purpose of monitoring the moisture is to ensure optimum moisture for healthy growth of *Epipremnum aureum*. Soil moisture was potentially affected by a number of factors such as weather conditions and humidity of air which could affect loss of moisture from soil via evaporation [22]. In view of variable weather conditions, humidity and evapotranspiration by plant,
the watering frequency was adjusted accordingly to prevent wilting of plants. This led to variability of soil moisture shown in Table 2. The overall increasing trend of soil moisture was largely attributed to increasing watering frequency as the plants showed signs of wilting in the first two weeks of transplant, in addition to the hot weather in the subsequent weeks.

At the same watering frequency and volume, it was found that generally soil contaminated with higher amount of crude oil tended to retain slightly more moisture as crude oil had been reported to slow down evaporation of water from soil [18].

Table 2. Soil moisture contents.

| Week | 5% Crude Oil | 10% Crude Oil | 15% Crude Oil |
|------|--------------|--------------|--------------|
|      | Without Fertilizer | With Fertilizer | Without Fertilizer | With Fertilizer | Without Fertilizer | With Fertilizer |
| 1    | 25.63        | 26.73        | 25.31        | 28.68        | 27.14        | 23.41        |
| 2    | 33.69        | 39.21        | 37.96        | 41.57        | 45.46        | 48.11        |
| 3    | 35.51        | 32.88        | 38.50        | 32.51        | 43.21        | 46.26        |
| 4    | 41.81        | 32.63        | 49.06        | 39.06        | 58.73        | 54.44        |
| 5    | 43.34        | 41.73        | 47.93        | 38.12        | 58.73        | 64.47        |
| 6    | 65.58        | 63.91        | 57.44        | 57.06        | 64.80        | 61.22        |

3.4. Limitation

The study has few inherent limitations. First, homogenous mixing of crude oil with soil might not have been attained though mixing was rigorously carried out. Second, watering though conducted in a controlled manner by spraying, might cause redistribution of crude oil in the soil. Third, soil was sampled at a fixed distance from the plant but the extent of root growth and penetration in the soil could be different, affecting the extent of phytoremediation. Fourth, the uptake hence the effect of fertilizer reaching the plant could not be tracked. Time was also a constraint in this study which did not permit longer experimental duration.

4. Conclusion

This study demonstrates that Epipremnum aureum can survive in soil contaminated with 5, 10 and 15% of crude oil and its ability to phytoremediate the contaminated soil. The rates of crude oil removal by Epipremnum aureum at all levels of contamination were increased by fertilizer application. This study contributes to the identification of Epipremnum aureum as a low-cost and hardy candidate for phytoremediation of soil contaminated with crude oil. This finding adds to the known ability of Epipremnum aureum to rhizofilter Co-60 and Cs-137 as well as its ability to clean indoor air [24, 25].

5. References

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