

# The Integrity of Biostimulant Efficiency of Mint Leaf (*Mentha piperita*) on Crude Oil Remediation in the Soil Environment

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## Abstract

*This research addresses the integrity of the biostimulant efficiency of mint leaf (*Mentha piperita*) on crude oil remediation in soil environments. The soil environment consideration in this investigation is loamy and some soil properties were examined; obtained data revealed a nitrogen value of 5.82%, phosphorus value of 46.91 mg/kg, potassium value of 417.51 mg/kg, and hydrogen-utilizing bacteria (HUB) value of 13.19 cfu/g for unpolluted loamy soil, whereas polluted soil with crude oil data are 1.02% nitrogen, 1.326 mg/kg phosphorus, 42.18 mg/kg potassium, and 81.6 cfu/g HUB. The properties of the biostimulant used revealed a nitrogen concentration of 6.93%, phosphorus of 52.271 mg/kg, potassium of 463.902 mg/kg, and HUB of 10.21 cfu/g for sun-dried mint leaf samples, whereas room-dried nitrogen value is 9.47%, phosphorus value is 74.113 mg/kg, potassium value is 552.542 mg/kg, and HUB value is 13.98 cfu/g. The density and kinematic viscosity of the crude oil used are 0.861 g/ml and 2.84 mm<sup>2</sup>/s. Crude oil remediation was monitored for a period of 35 days, and the percentage reduction in the total petroleum hydrocarbon (TPH) concentration was determined. Application of sun-dried biostimulant revealed TPH reduction values in terms of percentage removal within the range of 1.43% to 77.88% for intervals of 7 to 35 days with a 50 g dosage of remediant and 4.51% to 91.14% for 100 g dosage, whereas room-dried biostimulant application demonstrates 2.38% to 78.53% for 7 to 35 days of 50 g dosage and 8.41% to 92.29% for 100 g dosage of remediant. In the control sample, the data revealed 1.13% to 25.67% for the period of 7 to 35 days. This research revealed that the characteristics of mint leaf (*Mentha piperita*) possess the integrity of good biostimulant efficiency for use in treating soil contaminated with crude oil. However, the room-dried sample is higher in efficiency than the sun-dried, comparing percentage reduction of TPH concentration from 2.38% to 78.53% for intervals of 7 to 35 days of 50 g dosage against 1.43% to 77.83% for the sun-dried value. This investigation has demonstrated the significance of the biostimulant in crude oil remediation.*

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## INTRODUCTION

This study aims to provide a solution for the appropriate treatment options necessary for the effective restoration of soil contaminated by petroleum hydrocarbons. Several plant extracts have been reported in the literature [1–3]. This is because some bioremediants may be effective for the degradation of polluted soil, but because of the method of preparation and the proportion used, they may not be effective [4]. Additionally, applying mint leaves in bioremediation improves soil fertility

and enhances crop yields [5]. However, different technologies and plants have been used as biostimulants for the treatment of contaminated soil environments with crude oil or petroleum hydrocarbons, which have yielded positive results or solutions [6, 7]. Therefore, there is a need to test other plants and check the possibility of using them as bioremediants [8]. For any plant to be used as a bioremediant, its constituents must contain substances in high concentrations, such as total nitrogen, total phosphorus, total organic carbon, and potassium.

This study showcases the constituents of mint leaves and their suitability for use as bioremediants because of their nutrient availability found in them. This study projects mint leaves as a useful biostimulant. The effectiveness of the bioremediant was demonstrated by sampling the experimental setup and results obtained at the end of each sampling. The research shows how effective and useful this leaf can be in treating sites contaminated with petroleum hydrocarbons, as well as providing a high rate of soil restoration.

Various studies on technology, methods, and plant extracts for the remediation and restoration of soil contaminated with petroleum hydrocarbons are available in the literature. Where there is a population increase, the availability of food and other agricultural produce becomes necessary for a good standard of living of the populace [9]. Alternate sources of biostimulants that are more efficient and environmentally friendly are necessary. The use of modified and non-modified biostimulants for the removal of petroleum hydrocarbons and their derivatives has been widely reported [10–12]. Millions of microorganisms have been used for the effective remediation of petroleum hydrocarbons in different soil environments [13, 14]. Therefore, it is necessary to investigate the potential of mint leaves for the remediation of petroleum hydrocarbons in different soil environments. In the microbial-phytoremediation system, microorganisms convert pollutants, such as petroleum hydrocarbons, into substances that are easy for plants to directly absorb and utilize and plant roots can provide suitable places for microorganisms to survive [15, 16].

## **MATERIALS AND METHODS**

### **Conceptual Description of Parameters**

#### ***Bioremediant***

The following procedures were applied to obtain bioremediants as nanoparticles. The bioremediant (*Mentha piperita*) mint leaf was obtained from the Isiokpo Community in the Ikwerre Local Government Area of Rivers State, Nigeria. The mint leaves were placed in a polyethylene bag and transported to the chemical/petrochemical engineering laboratory. Every measurement was taken so that the mint leaf would not lose its properties, and it was subjected to room drying and some portion to sun drying. This method enhances the mitigation of moisture content, and once the mint leaf is dried properly, the obtained material is subjected to crushing to obtain nanoparticles. The nanoparticles of the mint leaves were analyzed experimentally for nitrogen content, phosphorus, potassium, bacterial counts, and fungal counts.

#### **Crude Oil**

The materials to be determined from the crude oil sample were total petroleum hydrocarbon (TPH) concentration, microbial isolation, and identification.

#### **Soil Sample**

The parameters that were determined from the soil sample were pH, electrical conductivity of the soil, total organic carbon, total nitrogen, total phosphorus, potassium, and microbial count.

#### **Experimental Setup**

There are various methods applied to achieve experimental setup, which include as follows.

1 g of the soil sample was measured and introduced into a reactor that received 100 ml of crude oil sample as a pollutant. Some reactors received 50 g dosages of the biostimulant containing room-dried

and sun-dried samples, whereas two different reactors received 100 g dosages of the biostimulant of the room-dried and sun-dried samples.

One kilogram of the loamy soil sample was measured and introduced into five plastic containers, in which two sets were labeled A1, A2, and B1, B2, and another set was labeled C. The first set, labeled A1 and A2, received 100 ml of crude oil and 50 g for A1 or 100 g for A2 of the bioremediant of sun-dried samples.

The second set, labeled B1 and B2, also received 100 ml of crude oil, 50 g of B1, and 100 g of B2 of the bioremediant of room-dried samples. The third set, labeled C, received zero bioremediation and 100 ml of crude oil. This sample was used as the control sample. Samples for analysis were taken at intervals of seven days to analyze the determination of TPH mitigation, as well as microbial counts for both bacteria and fungi in terms of hydrocarbon-utilizing bacteria and total fungi. The loamy soil type was weighed into bioreactors of fixed mass samples involving petroleum and varying quantities of mint leaves and was put into the bioreactors of loamy soil samples thoroughly mixed to ensure uniformity of concentration. The bioreactors containing the control samples A1 and A2 were left alone without any form of contamination. The experimental setup involved creating multiple bioreactors with varying concentrations of remediant.

#### **Bioreactor Setup**

- *A1*: 1 kg loamy soil + 100 ml of crude oil + 50 g sun-dried bioremediant (*Mentha piperita*)
- *A2*: 1 kg loamy soil + 100 ml of crude oil + 100 g sun-dried bioremediant (*Mentha piperita*)
- *B1*: 1 kg loamy soil + 100 ml of crude oil + 50 g room-dried bioremediant (*Mentha piperita*)
- *B2*: 1 kg loamy soil + 100 ml of crude oil + 100 g room-dried bioremediant (*Mentha piperita*)
- *Control*: 1 kg loamy soil + 100 ml of crude oil without bioremediation

## **RESULTS AND DISCUSSION**

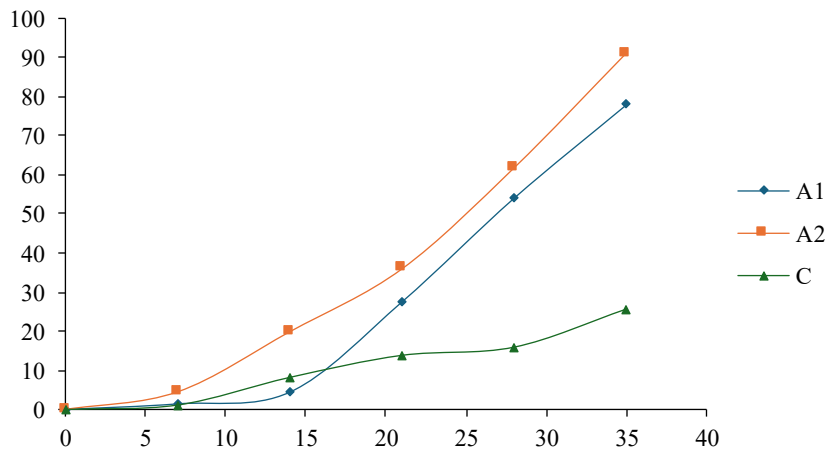
### **Effect of TPH Percentage Reduction**

The percentage removal of TPH was computed for both applications of bioremediants of room-dried and sun-dried samples introduced into different bioreactors. The total percentage removal of TPH and biostimulant efficiency occurred within 35 days of exposure. From the results, comparing A1 (50 g of sun-dried remediant) and A2 (100 g of sun-dried remediant), it is clear that the degradation of TPH was more effective in the bioreactor with a high deposit of the remediant and the efficiency of the bioremediation. This observation is also shown in B1 (50 g of room-dried remediant) and B2 (100 g of room-dried remediant). Again, the bioreactor with the room-dried deposit showed a high percentage of TPH degradation and higher biostimulant efficiency. This is a result of the hydrocarbon-utilizing bacteria of the different remediant (room-dried and sun-dried remediant).

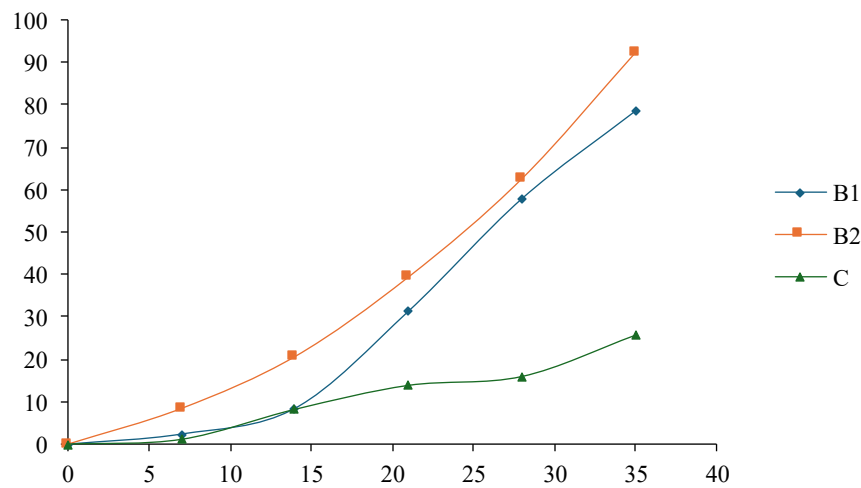
Figure 1 shows the degree of TPH removal in terms of percentage rating and the plot of TPH reduction against contact time for TPH degradation in bioreactors A1 and A2, and the control sample was monitored. The obtained results demonstrated the order of percentage of TPH removal as TPH A2 (100 g) > TPH A1 (50 g) > TPHC using a sun-dried sample of the bioremediant (mint leaf).

Figure 2 shows the comparison of TPH reduction in bioreactors B1, B2, and C upon the application of the bioremediant (mint leaf) in TPH remediation; the concentration of the bioremediant dosage influences the rate of TPH reduction in each bioreactor. The order of TPH removal was TPH B2 (100 g) > TPH B1 (50 g) > TPH C. In this case, the bioremediant used was dried, and microbial growth in each bioreactor was not induced by the inhibiting factors.

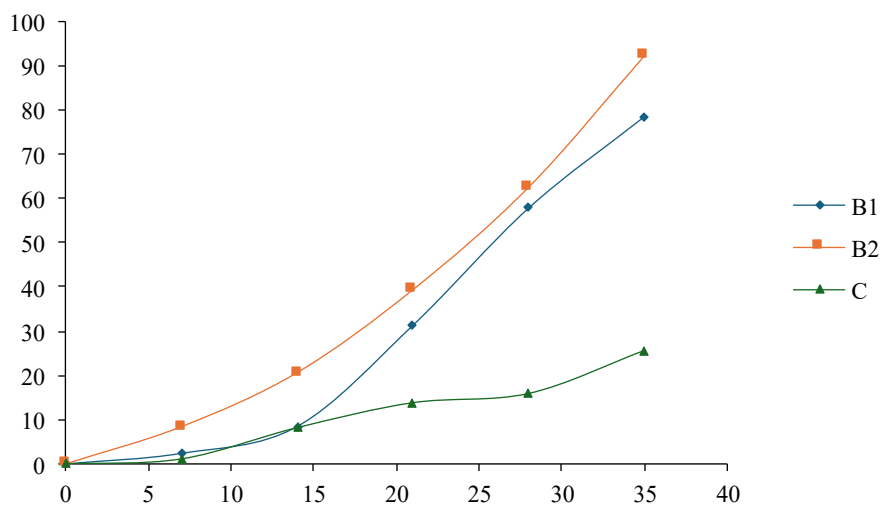
Figure 3 shows the comparison of the TPH removal by the application of both sun-dried and room-dried of the bioremediant, as well as comparing the performance with respect to the dosage mass concentration; the order of TPH removal was influenced by the bioremediant dosage mass concentration, which demonstrates the order of TPH B2 (100 g) > TPH A2 (100 g) > TPH B1 (50 g) >



**Figure 1.** Comparison of percentage removal of TPH versus time for sun-dried application of bioremediant (*Mentha piperita*), with control sample.



**Figure 2.** Comparison of percentage removal of TPH versus time for room-dried application of bioremediant (*Mentha piperita*), with control sample.

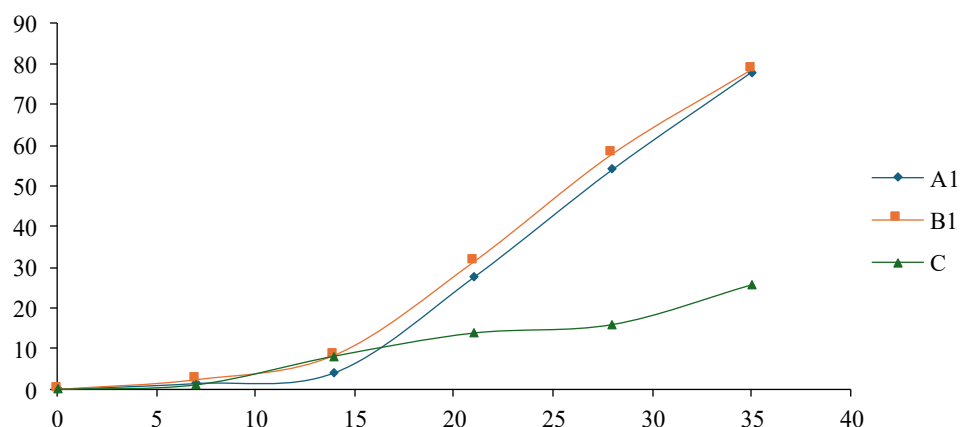


**Figure 3.** Comparison of percentage removal of TPH versus time for sun and room-dried application of bioremediant (*Mentha piperita*), with a control sample.

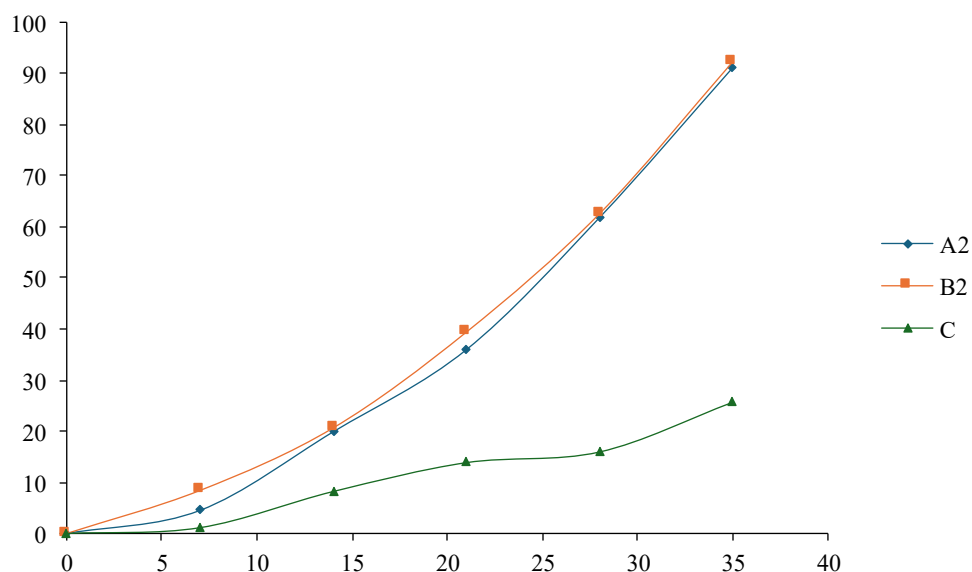
TPH A1 (50 g) > TPH C. The TPH removal percentage was monitored for 35 days, and the values obtained were 92.29% (B2) > 91.14% (A2) > 78.53% (B1) > 77.88% (A1) > 25.67% (C). Research has further revealed that the mint leaf is a good bioremediant and can be used in contaminant remediation or clean-up in polluted soil environments.

Figure 4 shows the comparison of the TPH percentage reduction in each bioreactor upon the effect of the bioremediant (mint leaf) introduced, which acts as a bioremediant; in this case, it is tested by comparing the TPH percentage removal with respect to the condition of preparation of 50 g dosage of sun-dried and room-dried samples. The results show that B1 (room-dried bioremediant) is greater than A1 (sun-dried bioremediant) and the control sample that does not receive the application of the nutrient (zero bioremediant).

Figure 5 shows the comparison of the TPH percentage removal upon the effect of dosage application, which is dependent on the sample preparation conditions of sun and room drying. The research revealed that the room-dried sample performed better than the sun-dried sample, which was integrated with the fact that some nutrients in the mint leaf were lost owing to the effect of sunlight. However, the variation in percentage removal was small.



**Figure 4.** Comparison of percentage removal of TPH versus time for sun and room-dried application of (50 g) dosage of bioremediant (*Mentha piperita*), with a control sample.



**Figure 5.** Comparison of percentage removal of TPH versus time for sun and room-dried application of (100 g) dosage of bioremediant (*Mentha piperita*), with a control sample.

## CONCLUSION

Based on the experiment conducted and the results obtained in this study, the following conclusions were drawn.

- The physicochemical properties of the remediant and remediant deposit levels affect their bioremediation.
- The analysis showed the percentage removal of TPH from the five reactors (A1, A2, B1, B2, and C) within a period of 35 days.
- The TPH removal from the reactors was 77.88% and 91.14% for the sun-dried remediant (*Mentha piperita*), 78.53% and 92.29% for the room-dried remediant, and 25.67% for the control.
- This result showed that the degradation of TPH was more effective in the bioreactor with a high deposit of the remediant and the room-dried remediant (*Mentha piperita*).
- The selected mint leaves (room-dried and sun-dried) (*Mentha piperita*) showed high potency and the possibility of being used as agents for bioremediation.
- The efficiency of the biostimulant for the different bioreactors was 52.88% for A1 (50 g of sun-dried remediant), 66.14% for A2 (100 g of sun-dried remediant), *Mentha piperita*, 53.53% for B1 (50 g of room-dried remediant), 69.20% for B2 (100 g of room-dried remediant), and 0.00% for the control with no remediant. (*Mentha piperita*).
- The results showed that remediants with high hydrocarbon-utilizing bacteria were more effective in substrate removal.
- Again, the higher the deposit of remediant, the more TPH is degraded.
- The effectiveness of the mint leaf in terms of percentage reduction of TPH was within the range of 52.88% to 69.20%, whereas the use of neem root in the same application revealed a percentage reduction of TPH up to 99.1%.

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