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Investigation on the Characteristics of *Chromolaena Odorata* (Awolowo leaf) in Petroleum Hydrocarbon Degradation in Loamy Soil Environment

Ukpaka Chukwuemeka Peter[1], Obinna Isaac Ikechi[2,*], Victor Chukwuemeka Ukpaka[3], Joy Chukwuemeka Peter Ukpaka[4], Abraham Peter Ukpaka[5]

Abstract

The application of Chromolaena odorata *(Awolowo leaf), processed into powder form, was used as a biostimulant for petroleum hydrocarbon degradation and mitigation in a loamy soil environment. In this investigation, the physicochemical and microbial characteristics of the sample were determined, as presented in this research, including pH, electrical conductivity (EC), total nitrogen (T.N), total phosphorus (T.P), potassium (K), total organic carbon, and P. fluorescence.* Corynebacterium *sp.,* Arthrobacter*,* Bacillus*,* Acinetobacter iwoffi*,* Flavobacterium *sp., and* Micrococcus roseus *were identified as bacteria, while* Aspergillus *sp.,* Aspergillus polyporicola*, and* Fusarium equiseti *were identified as fungi. Microbial populations of 1.38 × 10² cfu/g and 1.13 × 10² cfu/g were identified as bacteria and fungi in loamy soil, respectively, while 3.91 × 10⁶ cfu/g and 5.85 × 10⁴ cfu/g were identified from the biostimulant. The research revealed the effectiveness of the biostimulant in the mitigation of contaminated loamy soil, with total petroleum hydrocarbon (TPH) concentration reduced by 95–98% within 42 days for sun- and room-dried samples. However, the biostimulant of the room-dried sample was more effective than the sun-dried. This recommendation was based on the available nutrients present in the room-dried sample compared to the sun-dried, as well as the concentration of TPH after 42 days. This study further underscores the potential of* Chromolaena odorata *as an environmentally sustainable solution for bioremediation in hydrocarbon-contaminated soils.*

***Author for Correspondence**
Obinna Isaac Ikechi
E-mail: peter.ukpaka@ust.edu.ng

[1]Professor: Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, Rivers State, Nigeria.
[2]Research Student: Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, Rivers State, Nigeria.
[3]Research Student: College of Engineering, Computer Studies and Architecture, Department of Industrial Engineering, Lyceum of the Philippines University Cavite, Philippines.
[4]Research Student: College of Allied Medical Sciences, Department of Pharmacy, Lyceum of the Philippines University Cavite, Philippines.
[5]Research Student: Department of Computer Engineering, Lyceum of the Philippines University Cavite, Philippines.

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INTRODUCTION

The Niger Delta region has experienced severe crude oil soil pollution as a result of the current activities of illegal refineries, often known as Kpofire, and crude oil pipeline vandalism [1-4]. Agriculture will suffer over time, endangering the health of plants and other living things, if a solution or control mechanism is not proposed [5]. This is because soil degradation from crude oil has a harmful effect on the environment. Both the concept of using plants to accelerate the repair of contaminated soil environments and the application of bioremediation are not new [6]. The *Chromolaena odorata* (Awolowo leaf) that was acquired from the Mgbuitanwo community in the Emohua Local Government Area of Rivers State, Nigeria, was in fact examined to determine the

significant physiochemical features in connection to phytoremediation of polluted loamy soil environment. This study will show that *Chromolaena odorata* (Awolowo leaf) has the qualities needed to function as a bioremediant. The analysis of the *Chromolaena odorata* will show the efficiency of the samples that were sun and room dried as well as the rate in terms of percentage removal of the petroleum hydrocarbon from the contaminated loamy soil [7-11]. In terms of the rate of petroleum hydrocarbon degradation, the maximum specific rate of petroleum hydrocarbon degradation, as well as the constant parameter of dissociation or equilibrium, the inquiry will show the biokinetics parameters of the functional coefficient [12].

Experimental Design Samples of Petroleum Hydrocarbon (crude oil), Loamy Soil, and Remediant were collected. Crude oil and loamy soil's initial hydrocarbon concentrations before contamination were analyzed. *Chromolaena odorata* (Awolowo leaf) was prepared to produce powder form as the final raw material for testing and setting up. By combining a specific amount of loamy soil with a specific amount of petroleum hydrocarbon and varying the mass of the remediant in accordance with the experiment's design, each bioreactor produced samples that were taken at intervals of one week (7 days) for TPH analysis, physicochemical analysis, and microbial analysis.

MATERIALS AND METHODS

Materials

Awolowo leaf (*Chloromolaema odorata*), loamy soil, crude oil, plastic containers, a shovel, and a hand trowel were all employed in this study.

Collection of Samples

The department of Chemical/Petrochemical Laboratory received a sample of loamy soil from the Agricultural Farm Rivers State University, Nkpolu Oroworukwo in Port Harcourt. At the same time, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, the soil science department put a portion of the soil sample to analysis. The soil sample was prepared and set up for the experiment.

The Shell Petroleum Development Company (SPDC)-owned soil site in Ogoni was where the druse oil sample was taken. The collected sample was also sent to Rivers State University's Department of Chemical and Petrochemical Engineering Laboratory in Port Harcourt for evaluation. The crude oil used is specially prepared for the experiment and is of Bonny light medium quality. As a bio-remediant, the Awolowo leaf (*Chloromolaema odorata*) was procured from the Mgbuitanwo community in the Emohua Local Government Area of River State. The Awolowo leaf (*Chloromolaema odorata*) was prepared for the experimental setup and the obtained bio-remediant was brought to the same Department of Chemical/Petrochemical Engineering Laboratory.

Tools/Equipment Used

The following instruments and equipment were utilized for this study: dish, measuring cyclinder, pH meter, thermometer, and weighing scale.

Prepared Loamy Soil

A variety of physio-chemical characteristics, including pH, electrical conductivity, particle size, porosity, phosphorus, nitrogen, and organic matter, were determined for the loamy soil taken from the River State University Nkpolu Oroworukwo, Port Harcourt Farm. For this research, the loamy soil was cleaned of stones, grass, and other contaminants and made ready for usage.

pH Evaluation

Based on hydrogen ion concentration, pH is a measurement of the acidity of the soil and is technically defined as the negative logarithm of hydrogen ion concentration. A total of 15g of soil is weighed into two identical extraction cups. To prevent moisture loss, cups are sealed after being weighed. Thirty milliliters of deionized water is then added to each cup, sealed, and shaken for a short time. The cap is

taken off to provide the solution at least 30 minutes to acclimatise to the atmosphere. The pH meter's range is between pH 7 and pH 4. The electrode is inserted into the slurry and the pH is measured to the closest 0.01 while the slurry is being gently stirred. The electrodes are cleaned with de-ionized water in between samples.

Electrical Conductivity

It is a measurement of the dissolved substance in water that is connected to the substance's capacity to carry an electrical current, and it is expressed in mS/cm. The electrical conductivity of the material increases with the amount of dissolved material in the soil sample. A meter and probe can be used to determine the electrical conductivity. Two metal electrodes spaced one centimeter apart make up the probe. An electrical current flow through the aqueous sample as a result of applying a constant voltage across the electrodes. Since the number of dissolved ions in the soil is inversely correlated with the current flowing through the soil sample,the sample is more conductive and therefore has a higher conductivity reading when there is a higher concentration of dissolved salt/ions [13].

Size of Particles

Ultimately, soil is made up of a variety of sized and shaped soil particles. The goal of a particle size analysis is to divide these particles into distinct size ranges and calculate the relative weight proportion of each size range [14]. To determine soil suspension and periodically measure the density of the suspension, the method uses sieving and sedimentation techniques based on the application of Stokes' law.

Phosphorus

Use Bary No.1 solution on soil with a pH of 7.5 or lower to extract only absorbed forms of phosphate. The reaction with ammonium molybdate and the development of the blue color are used as the basis for a colorimetric measurement of the extracted phosphorus. The amount of phosphorus removed from the soil is directly proportional to the compound's absorbance, which is measured at 882 nm in a spectrometer.

Nitrogen

2g scoop of soil is mixed with 30 milliliters of calcium sulfate extraction solution for 15 minutes, and the amount of nitrogen in the extract is measured using a LaChat QuickChem 8500 Flow Injection Analyzer using the cadmium reduction method. The results are given as nitrates-nitrogen (NO_3-N) in soil parts per million.

Calcium

By combining a 1g scoop of air-dried soil with 10ml of standard ammonium acetate, pH 7, and shaking the mixture for five minutes, potassium can be extracted from the soil. The available potassium is evaluated by examining the filtered extract on an atomic adsorption spectrometer set on emission mode at 776nm. The results are reported as part per million (ppm) of potassium (k) in the soil.

Organic Content

This test is used to determine the organic content of soils. The organic material content is the ratio, stated as a percentage, of the mass of organic matter given mass of soil to the mass of the dry soil solids. Organic matter influences many of the physical, chemical and biological aspects of soil.

Moisture Content

The approach is based on removing soil moisture by oven-drying a soil sample until the weight remains consistent. The moisture content (%) is determined from the sample weight before and after drying. The approach covers the laboratory determination of the moisture content of a soil as a percentage of its oven-dried weight. The approach may be applied to fine, medium and coarse-grained soils for particle sizes from 2mm to >10mm.

Organic Matter

The most frequent method used to assess the quantity of organic matter present in a soil sample is by measuring the weight lost by an oven-dried (105 ºC) soil sample when it is heated to 400 ºC, this is known as loss on ignition. In laboratories, it is more typical to measure the total carbon content of the soil by dry combustion and elemental analysis. The amount of carbon measured can be turned into an estimated amount of organic matter.

Crude Oil Analysis

The crude oil sample used was classified as bonny light medium.Its microbial isolation and identification was also done. The physio-chemical properties of the selected parameters was also done and following parameters were considered which include, pH, temperature, electrical conductivity, total dissolved solids and total petroleum hydrocarbon initial concentration. Standard procedures were utilized for the measurement of total dissolved solid, pH, electrical conductivity and temperature of the crude oil sample and gas chromatography was employed in sampling the individual hydrocarbon concentration, which was totalled together to obtain the total petroleum hydrocarbon [15].

Gas Chromatography

An equipment used for crude oil analysis. It is an analytical tool that examines the content of several components in a sample. The analysis conducted by gas chromatograph is called gas chromatography. The principle of gas chromatography, the sample solution injected into the instrument enters a gas stream which carry the sample into a separation tube known as column (Helium or nitrogen is employed as the carrier gas). Inside the column, the various parts are separated. The detector monitors the quantity of components that escape the column. To measure a sample with uncertain concentration, a standard sample with a known concentration is introduced into the device. When compared to the test sample, the standard sample's peak retention time and area are used to compute the concentration.

pH Analysis for Crude Oil

Using a universal indicator strip, dip one end of the strip in the oil that is tested for pH level. Set the universal indicator on a dry surface for 60 seconds to allow the pH reading to take place. Compare the indicator paper's color shift to the pH chart that was included with the paper's package. To determine the precise pH match, compare the new color of the indicator paper to the chart.

Temperature Analysis for Crude Oil

By dipping a basic thermometer into the crude sample and reading the result, one can determine the temperature of the sample.

Total Dissolved Solid for Crude Oil

Make sure the instrument is clean and demineralized water is being used. To obtain the crude TDS, insert the probe into the sample being used.

Microbial Examination

It includes using biological, biochemical, or chemical techniques to find, recognize, or count microorganisms.

Identification and Isolation of Microorganisms

Bacterial isolation uses a variety of plating procedures, including spreading, pouring, streaking, and serial dilution, to isolate one strain of bacteria from a mixed culture of bacteria. Both the solid nutrient medium and the liquid broth medium can support bacterial growth. Bacterial isolation is a valuable approach for investigating and categorizing bacteria based on microscopic traits like color, size, and form. For the isolation and growth of bacteria, the serial dilution technique is widely used. Use the serial dilution method to sequentially dilute the bacterial culture in additional test tubes. Following the serial

dilution, add 1 ml of the sample to the next test tube in a sequence of 10x, 100x, 1000x diluted, and so on. Following the serial dilution of the bacterial solution, we can inoculate the bacterial culture by pouring, spreading, or streaking. Serial dilution makes it easy to isolate a bacterium from a smaller bacterial population [16, 17].

Microorganism Identification

Characterizing the diversity in microbial ecology is aided by the identification of microbes. Isolation, detection, and study of microorganisms are the main objectives. It also helps in identifying the organism down to the species or genus level, which can help in figuring out whether the organism is good or bad and if it causes diseases [18].

Microbial Culture

In order to obtain pure cultures, develop and measure microbial cells, nurture and identify microorganisms, among other things, several microbiological studies need culture media. Chemicals that aid in the growth, survival, and well-being of microorganisms are known as microbiological growth media. The growth medium contains nutrients, growth regulators, energy sources, buffer salts, minerals, metals, and suspending agents. The most often used microbe growth media are nutrient broths and agar plates. Only some types of bacteria can grow in selective media. Differential media, often referred to as indicator media, are used to separate one strain of microorganism that grows on a medium from another simply culture [19]

The first step towards isolation is the ability to culture a germ in a lab. In nature, microbial colonies live with a range of other cell types rather than isolating themselves by species. In a lab, these populations can be divided into pure cultures. Pure cultures, which only contain one species of bacterium, are excellent for examining their morphological, biochemical, and cultural traits.

The Spread Plate Culture Method

One of the most used techniques for isolating bacteria is spread plate culture. In order to easily count and separate microorganisms, it entails plating a sample mixture that contains them. A spread plate that works well will have a specific number of isolated microbial or bacterial colonies spread out evenly across the plate. This procedure involves using a sterilized L-shaped glass rod or spreader to apply a thin coating of a serially diluted sample (mixed culture) onto hardened agar plates while the agar plate is rotating on a platter. According to the hypothesis underlying this technique, single cells are distributed over the agar media's surface while the media plate is rotating. In the diluted samples, some of the cells will be sufficiently spaced apart from one another for colonies to grow on their own [20].

Pour-Plate Procedure

The most popular method for determining the total viable count is the pour-plate approach. This technique has the benefit that it can be used to test for bacterial contamination in food without the need for pre-made plates. The mixed culture is serially diluted during the pour-plate method using a loop or pipette. A predetermined volume of the diluted sample is placed in a Petri dish, which is then filled with molten agar that has been cooled to 45°C. When the agar is added, the lid is replaced. The plates are then gently rotated in a circular motion to ensure uniform microbe distribution. Repeat this process until all dilutions have been plate-tested. It is simple to carry out. Due to the huge sample volume, it will be able to detect lower levels than the surface spread method. In comparison to the spread plate and streak plate techniques, the pour plate method requires more preparation time. When heat-sensitive microorganisms come in contact with heated agar, they become nonviable.

Streak-Plate Technique

The most used technique for isolating colonies from microbial culture is the streak plate method. The technique works by reducing the concentration of bacteria and dilution of their number. It can be used by microbiologists to recognize and isolate particular bacterial populations. A colony is a group of

microorganisms that may be seen. All of the bacteria in a colony originate from the same bacterial cell. Single colonies are hence referred to as 'pure' colonies. A different plate is used to transfer the isolated pure culture of one kind of bacterium by two different streak plate techniques.

- *The T-streak method:* The T-streak method consists of three parts: an upper half, two equal-sized lower sections, and a lower segment. The upper portion of the plate receives the initial inoculum. From the top to one of the lower segments, then from that lower area to the next segment, bacteria are transferred.
- *The quadrant method*: It includes streaking four areas of the same size.
- *The continuous streaking technique*: It involves inoculating the top half of the plate, rotating it 180 degrees, and then inoculating the bottom half of the plate without sterilizing the loop or transferring bacteria from the area before it.

There are two techniques mentioned here for Petroleum Hydrocarbon Degradation in olamy soil environment.

Setting up an Experimental Layout

The crude oil (also known as petroleum hydrocarbon), loamy soil, remediant (Awolowo leaf), and plastic containers are used as reactors in the experimental layout set-up for this study. The experimental layout was divided into layouts A, B, and C, each of which contains petroleum hydrocarbon, loamy soil, and remediant that has been dried in a different environment. Layout C contains petroleum hydrocarbon and loamy soil that has not been treated with remediant. The following examples show how the experimental layout was set up.

Loamy soil [LS] + petroleum hydrocarbon [H] + enzyme catalyzed by remediant room dried [M].

For layouts A and B, the amount of Petroleum hydrocarbon [H] used was constant at 100ml, the amount of loamy soil used was also constant in mass, and the amount of remediant used ranged from 25g to 100g at intervals of 25g. However, there was no remediant added to layout C. The designs are

- Room-dried Remediant: [H]100ml + [LS]2kg + [E]25g For Reactor A1
- Room-dried Remediant: [H]100ml + [LS]2kg + [E]50g For Reactor A2
- For Reactor A3 of Room-dried Remediant, use [H]100ml + [LS]2kg + [E]75g.
- Room-dried Remediant: [H]100ml + [LS]2kg + [E]100g For Reactor A4
- Sun dried remediant, [H]100ml + [LS]2kg + [E]25g for Reactor B1
- Sun-dried Remediant, [H]100ml + [LS]2kg + [E]50g for Reactor B2
- Sun-dried Remediant, [H]100ml + [LS]2kg + [E]75g for Reactor B3
- Sun-dried Remediant, [H]100ml + [LS]2kg + [E]100g for Reactor B4
- For Control Reactor C, there is no remediant added; instead, natural remediation takes place. [H]100ml + [LS]2kg.

Experimental Approach

The following experimental techniques were employed in the conduct of this study, including the measurement of 2kg of loamy soil and its introduction into nine (9) set up bioreactors. Eighteen bioreactors were separated into two groups, with one serving as the control. A1, A2, A3, and A4 are the labels for each of the four bioreactors in one group. B1, B2, B3, and B4 are the labels for the control group.

2kg of loamy soil, 100ml of crude oil (bonny light medium), and the bio-remediant mass of 25g, 50g, 75g, and 100g added to reactors A1, A2, A3, and A4 accordingly for sun drying were all added to each bioreactor (batch reactor). 2kg of loamy soil, 100ml of crude oil (bonny light medium), and 25g, 50g, 75g, and 100g of bio-remediant, respectively, were added to the bioreactors (batch reactors) of B1, B2, B3, and B4. For easy interaction between the microorganism and the crude oil, the set-up bioreactors

were injected with a small volume of distilled water (50ml), and mixed materials in each bioreactor were thoroughly stirred. This mechanism also enhanced non-inhibiting action of the microbes on the crude oil degradation.

Gas chromatography (GC) analysis was used to estimate the initial concentration of the total petroleum hydrocarbon after mixing in addition to other crucial factors. In the control, no bio-remediant was added, and the bioreactor setup was left to experience the normal degradation of petroleum hydrocarbons. After seven (7) days, samples were taken to measure total petroleum hydrocarbon (TPH) degradation, pH, temperature, electrical conductivity, total organic carbon, total nitrogen, phosphorus, and potassium, as well as total heterotrophic bacterial and total heterotrophic fungal counts. For a total of six weeks, the sampling steps were carried out periodically at a 7-day (1-week) interval. The samples were tested as previously mentioned to determine how the total petroleum hydrocarbon, physio-chemical properties of the chosen component, and microbiological analyses would change as the biomass increased. By connecting the data from the experimental setup to the mathematical theories of first order kinetics, second order kinetics, Michaelis-Menten kinetics, and Monod kinetics, all in terms of the rate model of crude oil degradation under the influence of a bioremediant (Biodegradation Model rate), the data was further developed.

RESULTS AND DISCUSSION

The results obtained from this research are presented in figures and tabular form.

Result on Physiochemical Parameters in Soil, Remediant, Microbial Count, and Microbial Characterization.

The Physiochemical Analysis of Room and Sun Dried Remediant and Soil Sample are presented in Table 1.

Table 1. Physiochemical analysis of room and sun dried of remediant and soil sample.

Parameters T.N(%)	**pH**	**E.C (uS/cm)**	**Temp (ºC)**	**T.P. (Mg/Kg)**	**TOC (%)**	**K (Mg/Kg)**
RD Awolowo Leaf *(Chlromoleana odorata)* 0.98	6.28	210.6	25.8	37.570	8.6	140.9
SD Awolowo Leaf *(Chlromoleana odorata)* 0.75	6.11	193.6	26.3	28.983	7.1	133.6
Loamy Soil 0.17	6.74	120.3	25.4	8.168	2.57	26.3

When comparing the *Chloromolaena odorata* samples that were dried in a room and those that were dried in the sun, Table 1 displays the concentration of the remediant's value. The room-dried samples had a pH of 6.28, an electrical conductivity of 210.6 S/cm, a temperature of 25.8 ºC, a total phosphorus content of 37.570 mg/kg, an organic carbon content of 8.6%, a potassium content of 140.9 mg/kg, and a total nitrogen content of 0.98%. According to the sun-dried remediant's results, the pH value is 6.11, electrical conductivity is 193.6 S/cm, the temperature is 26.3 ºC, the total phosphorus content is 28.983 mg/kg, the total organic carbon is 7.1%, the potassium value is 133.6% and the total nitrogen is 0.75%.

Table 2. Characterization of fungi isolated and identified in soil (loamy soil) used for investigation.

Fungi Vogues Isolated Proskaver	**Gram Staining**	**Motility**	**Methyl Red**	**Indole**	**Citrate Utilization**	**Sugar Fermentation**	**And Identified in Soil**
Aspergillus Sp	+	+	-	-	+	+	-
Aspergillus flavies	-	+	-	+	-	+	+
Aspegillus sydorii	+	-	+	-	+	-	+

Aspergillus polyporicola	+	+	+	+	+	+	+
Fasartium equseti	-	+	+	+	-	+	+

Similarly, the physiochemical parameters of the loamy soil sample were examined and showed pH of 6.74, electrical conductivity of 120.3 µs/cm, temperature of 25.4°C, total phosphorus of 8.168 mg/kg, total organic carbon of 2.57%, potassium of 26.3 mg/kg and total nitrogen of 0.17%.

To confirm the presence and absence of *Aspergillus sp., Aspergillus flavus, Aspergillus sydorii, Aspergillus polyporicola*, and *Fusartium equiseti* in the loamy soil sample, the results of the fungal characterisation are shown in Table 2. Gram staining, motility, citrate utilization, and fermentation tests revealed the presence of *Apsergillus sp*., while methyl red and indole tests revealed its absence. When tested for motility, the indole test, sugar fermentation, and *Vogues proskaver, Aspergillus flavus* was found; however, it was not present when tested for gram staining, methyl red, or citrate utilization. Gram staining was used to confirm the presence of *Aspergillus sydorii*.

Motility, indole tests, and sugar fermentation were not present, however methyl red, citrate utilizations test, and *Vogus proskever* were. Gram staining, motility, methyl red, indol, citrate utilization, sugar fermentation, and vogus proskaver tests all revealed the presence of *Aspergillus polyporieola*. When tested for motility, methyl red indole, sugar fermentation, and *vogus proskaver, Fusartium equiseti* was found to be present, but it was not present when the tests for gram staining and citrate utilization were performed.

Table 3. Microbial Population of Bacterial and Fungal isolated and identified from Loamy Soil ample.

Sample	**Bacterial Count**	**Fungal Count**
Soil (Loamy)	1.38×10^2	1.13×10^2

Table 3 results obtained show more of bacteria count when compared with fungi count. The concentration of bacteria is 1.38×10^2 cfu/g, while the concentration of fungi is 1.13×10^2 cfu/g.

Table 4. Microbial Population of Bacterial and Fungal in Awolowo leaf *(Chlromoleana odorata)*.

Sample	**Bacterial Count (cfu/g)**	**Fungal Count (cfu/g)**
RD Awolowo leaf (*Chlromoleana odorata*)	3.91×10^6	5.85×10^4
SD Awolowo leaf (*Chlromoleana odorata*)	3.46×10^6	4.92×10^2

The remediant (*Chlromoleana odorata*), which is available in powder form, contains all of the heterotrophic bacteria that have been isolated and identified, as shown in Table 4. When dried in a room, the microbial population is 3.91 x 106 cfu/g for bacteria and 5.85 x 104 cfu/g for fungi, compared to 3.46 x 106 cfu/g for bacteria and 4.92 x 102 cfu/g for fungus when dried in the sun.

The bacteria characterization for the soil sample is shown in Table 5. In the loamy soil sample, the following bacteria were found and characterized: *P. fluorescens, Corynebacterium, Arthrobacter, Bacillius sp., Acinetobacter iwoffi, Flavobacterium sp.,* and *Micrococcus roseus*. When tested for colony form, round cell shape, white colony color, smoothness, absence of the indole test, starch hydrolysis, glucose fermentation, nitrate to nitrite test, citrate test, absence of gelatin liquefaction, presence of methyl orange, and catalase test, *P. flourescene* was irregular. Corynebacterium had irregular colony form, bacilliform cells, light yellow colony color, smoothness, and presence for the indole test, starch hydrolysis, glucose fermentation, and nitrate to nitrite conversion. Present in the catalase test, absent in the methyl red test, and present in the gelatin liquefaction test. When tested for colony form, bacilliform cell shape, light yellow colony color, smoothness, presence in the indole test, starch hydrolysis test, glucose fermentation test, nitrate to nitrite test, citrate test, gelatin liquefaction test, absence in the methyl orange test, and presence in the catalase test, Arthrobacter was irregular. In

terms of colony form, bacilliform for cell shape, light yellow for colony color, rough for smoothness, and presence for the indole test, starch hydrolysis, glucose fermentation, nitrate to nitrite test, absence for the citrate test, absence for the gelatin liquefaction, presence for the methyl red test, and presence for the catalase test, *Bacillius sp.* appeared irregular.

Table 5. Bacteria characterization in soil.

Parameters	**Microorganisms Isolated and Identified in the Soil**						
	P.Fluoreseene	***Conynebacterium sp***	***Arthrobacter***	***Bacillius sp***	***Acinetobacter twiff***	***Flavobacterium sp***	***Micrococcus roseus.***
Colony form	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Cell shape	Round	Bacilliform	Bacilliform	Bacilliform	Bacilliform	Bacilliform	Eliptical
Colony order	White	Light yellow	Light yellow	Light yellow	Light yellow	White	Light yellow
Smoothness	Smooth	Light yellow	Light yellow	Roung	Roung	Smooth	Roung
Indole test	+	-	-	+	+	+	-
Hydrolysis of starch	+	+	+	+	+	+	-
Glucose fermentation	+	-	+	+	+	-	-
Nitrate to nitrate	-	+	+	+	-	+	-
Citrate test	+	-	+	-	+	-	-
Gelatin liquifaction	+	+	-	-	+	+	+
Methyl red	+	+	+	+	+	-	-
Catalase test	+	+	+	-	-	+	-

Acinetobacter iwoffi became visible, irregular in colony form, bacilliform in cell shape, light yellow in colony color, rough in smoothness, indole test negative, starch hydrolysis positive, glucose fermentation negative, nitrate to nitrite negative, citrate positive, gelatin positive, methyl positive, and catalase negative. When tested for colony form, bacilliform cell shape, white colony color, smoothness, presence for the indole test, starch hydrolysis test, glucose fermentation test, nitrate to nitrite test, absence for the citrate test, gelatin liquefaction test, presence for the methyl orange test, and presence for the catalase test, Flavobacterium sp. was found to be irregular.

When given colony form, Micrococcus roseus displayed irregularities. Its cells had bacilliform shapes, light yellow colonies, and rough surfaces. For smoothness, absent for indole test, present for hydrolysis of starch, absent for glucose fermentation, present for nitrate to nitrite, present for citrate test, absent for gelatin liquefaction, present for methyl orange, and present to catalase test.

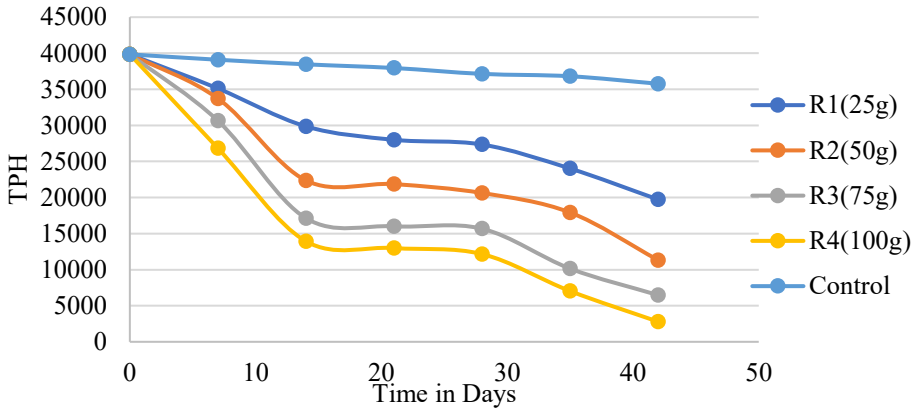

Figure 1. TPH concentration versus effect of time on remediant for bioreactor R_{R1}, R_{R2}, R_{R3}, R_{R4} and control of room dried.

Result Demonstrating Total Petroleum Hydrocarbon Degradation

The degradation of the total petroleum hydrocarbon in R_{R1}, R_{R2}, R_{R3}, R_{R4}, and the control reactor for room-dried application of *Chloromolaena odorata* leaf is represented in Figure 1.

The sun-dried application of *Chloromolaena odorata* leaf is presented in Figure 2 with reactors R_{S1}, R_{S2}, R_{S3}, and R_{S4} with the same control sample. The TPH degradation in each reactor was monitored and the results were recorded as samples was taken every 7 days.

Figure 1 shows the performance of Room dried bio-stimulant sample of *Chloromolaema odorata* used as a nutrient for purpose of catalyzing the process of degradation of TPH in each reactor. The different samples reactor is labeled R1, R2, R3 and R4 with control sample. The result demonstrates the significance of *Chloromolaema odorata* in enhancing TPH remediation in a polluted loamy soil environment. The investigation that shows that the effectiveness in the TPH remediation is dependent on the degree of dosage concentration in each bioreactor. The rapid order of remediation of TPH is expressed as R4 (TPH) > R3 (TPH) > R2 (TPH) > R1 (TPH) > Control Sample (TPH).

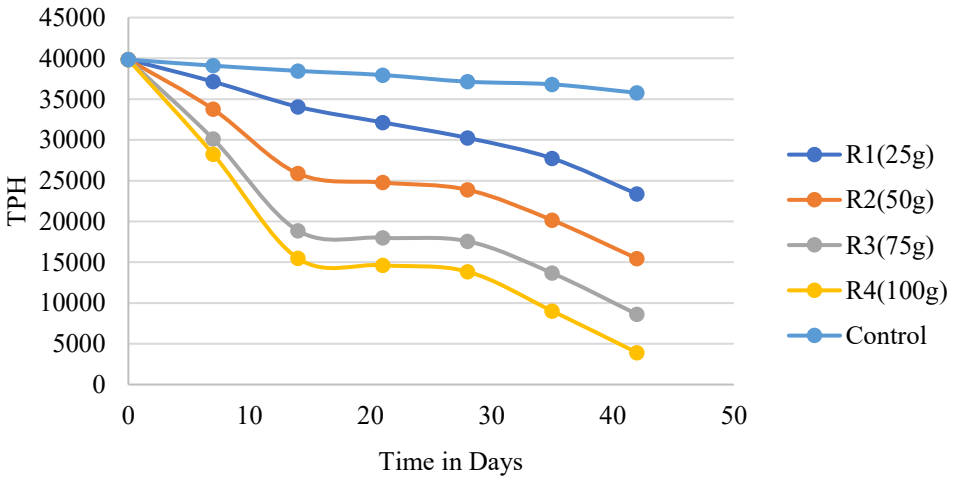

Figure 2. TPH Concentration Versus Effect of time on Remediant for Bioreactor R_{S1}, R_{S2}, R_{S3}, and R_{S4} and Control of Sun Dried.

Figure 2 displays the effectiveness of a Sun-dried Chloromolaema odorata bio-stimulant sample employed as a nutrient to catalyze the breakdown of TPH in each reactor. With a control sample, the several sample reactors are identified as R1, R2, R3, and R4. The outcome shows how important *Chloromolaema odorata* is for improving TPH cleanup in an environment with polluted loamy soil. According to research, the degree of dose concentration in each bioreactor determines how effective TPH remediation is. According to this formula, R4 (TPH) > R3 (TPH) > R2 (TPH) > R1 (TPH) > Control Sample (TPH) represents the quick order of TPH remediation.

CONCLUSION

The study demonstrates that Chromolaena odorata (Awolowo leaf) is an effective biostimulant for remediating petroleum hydrocarbon-contaminated loamy soil. The remediant accelerates microbial growth by providing essential nutrients, reducing the lag phase, and enabling efficient TPH degradation. The presence of specific fungi and bacteria was identified, which facilitated the bioremediation process without hindering microbial growth. The biokinetic parameters were determined, and it was found that room-dried remediant was more effective than sun-dried in TPH removal. The study highlights that Chromolaena odorata significantly enhances the restoration of contaminated soil, making it suitable for agricultural use, with a 95-98% hydrocarbon reduction within 42 days. Furthermore, the leaf, often discarded as waste, contains valuable components beneficial for plant growth.

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