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Phytoremediation of Crude Oil Polluted Sandy Soil using Plant Leaves: A Comparative Study

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Article Info	ABSTRACT
Corresponding Author: Nnadikwe Johnson E-mail: Nnadikwe.johnsonnnadikwe@cgrpng.org	This study evaluates the comparative efficacy of Jatropha curcas and Vernonia amygdalina leaf extracts in phytoremediating crude oil-polluted sandy soil. Leaf extracts containing P. aeruginosa, S. aureus, and E. coli were prepared via sun drying, room drying, and wet blending. Wet-blended extracts (10-40g, 40 days) achieved significant reductions: - Hydrocarbons: 2.40 µg/mL (Vernonia amygdalina), 2.11 µg/mL (Jatropha curcas). Lead (Pb): 0.99 µg/mL (Vernonia amygdalina), 0.72 µg/mL (Jatropha curcas). - Zinc (Zn): 0.71 µg/mL (Vernonia amygdalina), 0.51 µg/mL (Jatropha curcas). Predictive models ($R^2 > 0.875$) developed using Minitab software demonstrate the impact of leaf mass, treatment time, and soil pH on remediation. Results highlight the potential of these plant extracts as sustainable bio-remediation agents for crude oil-polluted sandy soil, offering an eco-friendly solution for environmental restoration. **Keywords:** Vernonia amygdalina, (Jatropha curcas,Soil, Remediation,Minitab.

INTRODUCTION

Background to the Study

Crude oil pollution is a pervasive environmental issue, posing significant threats to ecosystems and human health. Phytoremediation, a cost-effective and eco-friendly approach, utilizes plants to remove or degrade pollutants from contaminated soil. Bioaugmentation, the introduction of beneficial microorganisms, can enhance phytoremediation efficiencyCrude oil spills can devastate soil ecosystems, causing long-term damage to plant growth and microbial activity. Traditional remediation methods can be expensive and environmentally invasive. Phytoremediation offers a promising alternative, leveraging plant-microbe interactions to break down pollutant.

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. [Environmental Inquiry, 2017]. According to the United States EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances". Technologies can be generally classified as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site, while ex situ involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies are phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, leaves, etc,that help in enhancing the growth of the pollution-eating microbes within the medium (biostimulation). Depleted soil nitrogen status may encourage biodegradation of some nitrogenous organic chemicals, [Sims, 2006] and soil materials with a high capacity to adsorb pollutants may slow down biodegradation owing to limited bioavailability of the chemicals to microbes [O'Loughlin et al, 2000]. Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators.

However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium, zinc and lead are not readily absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil [Kris, 2012]. Bone char has been shown to bio-remediate small amounts of cadmium, copper, and zinc. [Huan Jing, 2007]. The assimilation of metals such as mercury into the food chain may worsen matters. Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bio accumulate these toxins in their above-ground parts, which are then harvested for removal. [Meagher, 2000]. In contrast to this situation, other contaminants, such as aromatic hydrocarbons as are common in petroleum, are relatively simple targets for microbial degradation, and some soils may even have some capacity to auto remediate, as it were, owing to the presence of autochthonous microbial communities capable of degrading these compounds. [Olapade, 2014]

The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

Key Concerns

The crude oil-polluted sandy soil in Ogoniland, Niger Delta, poses significant environmental, health, and economic risks, including:

1. Soil contamination: Widespread pollution of sandy soil due to oil spills, reducing fertility and affecting plant growth
2. Environmental degradation: Long-term damage to ecosystems and biodiversity, threatening local wildlife and ecosystems
3. Food insecurity: Impact on agricultural productivity and food availability, exacerbating poverty and malnutrition
4. Health risks: Potential health hazards due to exposure to pollutants, including carcinogenic hydrocarbons and heavy metals
5. Economic instability: Negative impact on local economies and livelihoods, perpetuating poverty and inequality

Problem Statement

The sandy soils of Ogoniland, Niger Delta, are severely contaminated with crude oil, posing significant environmental, health, and economic risks. Phytoremediation using

Jatropha curcas and Vernonia amygdalina plant leaves offers a promising solution, but the efficacy of these species in remediating crude oil-polluted sandy soils remains unclear. This study aims to compare the phytoremediation potential of these plant species, providing a sustainable solution for restoring soil fertility, promoting food security, and improving local livelihoods.

Objectives of the Study

This study aims to:

1. Evaluate the effectiveness of Jatropha curcas and Vernonia amygdalina leaf extracts in remediating crude oil-polluted sandy soils.
2. Compare the remediation potential of these plant species at different concentrations.
3. Develop a statistical model predicting the bioremediation process.

These objectives focus on comparing the phytoremediation efficacy of Jatropha curcas and Vernonia amygdalina in sandy soil

Contribution to UN Sustainable Development Goals (SDGs)

This research contributes to several UN SDGs:

1. SDG 2: Zero Hunger - By restoring soil fertility and promoting sustainable agriculture, this research can help improve food security and reduce hunger in communities affected by oil pollution.
2. SDG 3: Good Health and Well-being- By reducing the environmental impact of oil pollution, this research can help mitigate health risks associated with exposure to pollutants, promoting good health and well-being.
3. SDG 6: Clean Water and Sanitation- By remediating polluted soils, this research can help prevent water pollution and promote access to clean water and sanitation.
4. SDG 12: Responsible Consumption and Production- By promoting sustainable approaches to environmental remediation, this research can help reduce the environmental impact of human activities and promote responsible consumption and production patterns.
5. SDG 13: Climate Action- By enhancing understanding of sustainable remediation strategies, this research can help mitigate the impacts of climate change by reducing greenhouse gas emissions and promoting eco-friendly solutions.
6. SDG 15: Life on Land- By restoring ecosystems and promoting biodiversity, this research can help protect and conserve terrestrial ecosystems, promoting life on land.
7. By contributing to these SDGs, this research can help promote sustainable development, reduce environmental degradation, and improve human well-being.

Materials And Methods

This study used:

1. Plant Materials: Jatropha curcas and Vernonia amygdalina leaves
2. Soil Samples: Crude oil-polluted sandy soil
3. Laboratory Equipment: Incubators, Spectrophotometers
4. Statistical Software: R, Minitab

These materials will enable the evaluation of the phytoremediation potential of Jatropha curcas and Vernonia amygdalina in crude oil-polluted sandy soil

Ogoniland Overview

Ogoniland, a Niger Delta region, has been severely impacted by oil industry operations, with:

1. 1,000 km² area, 832,000 population (Ogoni people)

2. £30 billion worth of oil extracted, yet majority remain impoverished
3. Environmental degradation from spills, flares, and inadequate responses
4. Social unrest, MOSOP formed, led by Ken Saro-Wiwa, demanding autonomy and revenue share.

Study Site Description

The study site in Ogoni community, Niger Delta, has:

1. Surface spills from decommissioned oil wells and buried flare pits
2. Unknown/variable contamination age
3. Varying human disturbance (cultivation, excavation, compaction)
4. Large spill size (>1 ha)
5. No cattle grazing reported

These characteristics affect soil properties and bioremediation potential, informing the study design and analysis of phytoremediation using Jatropha curcas and Vernonia amygdalina in crude oil-polluted sandy soil

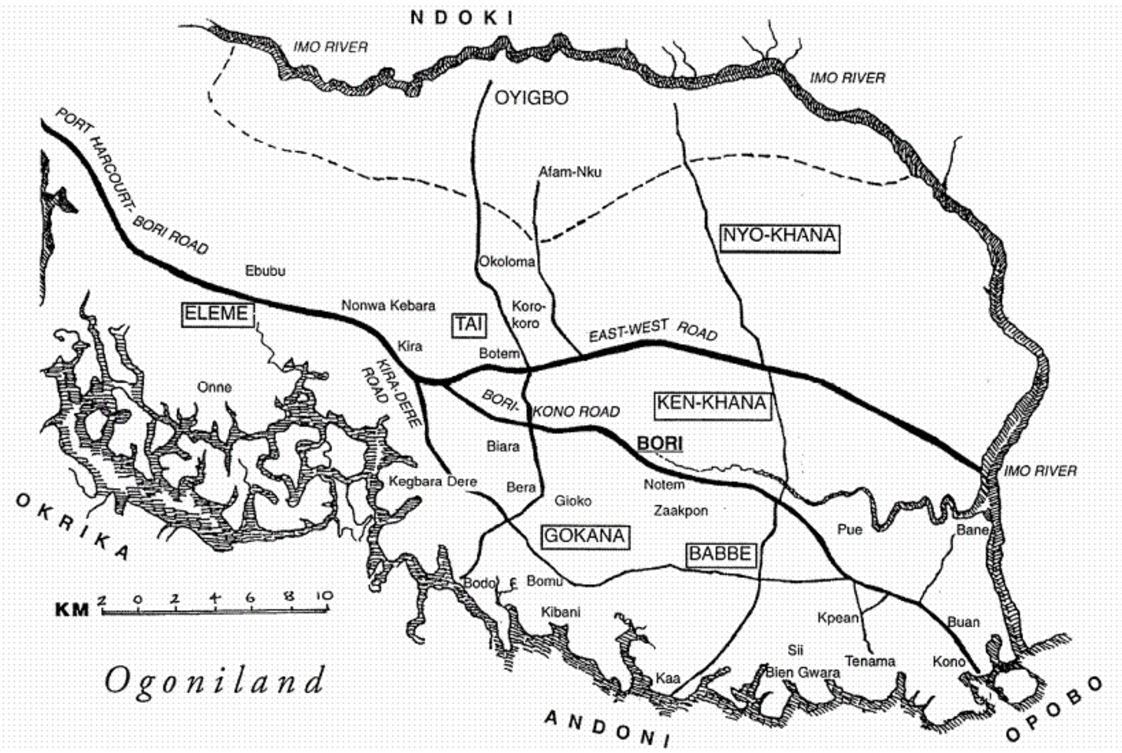

Fig .1: Map of Ogoni Land

Soil and Vegetation Sampling Methodology

Soil samples (sandy soil) were collected from contaminated plots (0-15 cm depth), sieved, and analyzed for:

1. %C and %N (LECO CNS-2000 Analyzer)
2. Petroleum hydrocarbons (mechanical shaking extraction, GC analysis)

This methodology supports the phytoremediation study using Jatropha curcas and Vernonia amygdalina in crude oil-polluted sandy soil.

Soil Analysis

pH, heavy metals (Zn, Pb), and hydrocarbon content will be analyzed in:

1. Unpolluted sandy soil
2. Crude oil-polluted sandy soil

3. Soil during bioremediation process

Leaf Sample Collection and Extract Preparation

1. Vernonia amygdalina and Jatropha curcas leaves collected from Umuabiara
2. Community, Imo State, Nigeria
3. Leaf extracts prepared using a juice extractor, using both juice and leftover chaff for analysis

Leaf Extract Preparation

Vernonia amygdalina and Jatropha curcas leaf chaff were treated in three ways:

1. Sun-dried
2. Room-dried
3. Wet-blended

These treatments evaluate the bio-remedial effects of leaf extracts on crude oil-polluted sandy soil.

Leaf Extract Analysis

Vernonia amygdalina and Jatropha curcas leaf extracts were analyzed for:

1. Physio-chemical composition (bio-remedial properties)
2. Microbial support (presence and stability of microorganisms)

These analyses assess the potential of leaf extracts in enhancing bioremediation of crude oil-polluted sandy soil.

Microbial Analysis

Materials used:

1. Sterilized equipment (conical flask, test tubes, Petri dishes)
2. Nutrient agar, broth, and distilled water
3. Vernonia amygdalina and Jatropha curcas leaf extracts

Procedure:

1. Sterilization of equipment (autoclave)
2. Preparation for microbial analysis of leaf extracts' support for microorganism growth.

Culture Preparation

1. Nutrient agar: 3g in 100mL distilled water (sterilized)
2. Nutrient broth: 5g in 500mL distilled water, distributed (20mL/test tube) and sterilized (autoclaved)

These cultures support microorganism growth for assessing Vernonia amygdalina and Jatropha curcas leaf extracts' bioremediation potential.

Serial Dilution Materials

1. 1mL syringe
2. 6 sterilized test tubes with nutrient broth
3. Vernonia amygdalina leaf extract
4. Bunsen burner (for sterilization)

Used for assessing microbial growth and bioremediation potential of leaf extracts.

Tenfold Serial Dilution

1. 1mL of leaf extract added to nutrient broth, mixed
2. 1mL transferred to next test tube, repeated for 20 tubes
3. Result: Decreasing concentrations (increasing powers of dilution) of Vernonia amygdalina and Jatropha curcas leaf extracts for evaluating effects on microorganisms.

Culture Preparation and Incubation

a. Nutrient agar (20mL) poured into Petri dishes, solidified
b. Inoculated with 1mL serially diluted broth culture
c. Incubated at 37°C to promote microbial growth

Evaluating effects of Vernonia amygdalina and Jatropha curcas leaf extracts on microbial growth and bioremediation potential

Plant Extract Preparation

Vernonia amygdalina and Jatropha curcas leaves extracted using:

a. Cold ethanol
b. Hot ethanol (80°C)
c. Cold water
d. Hot water (80°C)

Evaluating optimal solvent and temperature for extracting bioactive compounds for bioremediation

Preparation of Hot Ethanolic and Aqueous Extracts.

Hot Ethanolic and Aqueous Extract Preparation

a. 50g ground leaf powder extracted with 200mL ethanol/water (80°C, 1 hour)
b. Filtered, evaporated (45°C), and stored in aluminium foil
c. Reconstituted in 95% ethanol (250mg/mL, 4°C

Cold Ethanolic and Aqueous Extract Preparation

a. 50g ground leaf powder extracted with 200mL ethanol/water (48 hours, room temperature)
b. Filtered, evaporated (45°C), and reconstituted in 95% ethanol (250mg/mL)

The test organisms used were Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli.

Collection and Maintenance of Test Organisms

a. Maintained on nutrient agar slopes, stored in refrigerator
b. Standardized using Bauer et al. (1966) method

Antibacterial Assay

a. Agar dilution method
b. Minimum Inhibitory Concentration (MIC) determined using Oyagade et al. (1999) method.

Test Conditions

a. Incubation: 37°C, 18-24 hours
b. Medium: Mueller Hinton Agar (MHA)

Evaluating Vernonia amygdalina and Jatropha curcas leaf extracts' antibacterial potential.

Phytochemical Screening Results

a. Saponins: present (frothing test)
b. Alkaloids: present (Mayer's reagent/picric acid test)
c. Phenolics: present (ferric chloride test)
d. Tannins: present (potassium hydroxide test)
e. Steroids: present (sulfuric acid test)

These phytochemicals may contribute to bioremediation potential.

Phytochemicals Tested

a. Alkaloids
b. Tannins

c. Saponins
d. Phenols
e. Steroids
f. Flavonoids and glycosides

May contribute to antibacterial and bioremediation properties of Vernonia amygdalina and Jatropha curcas extracts.

Chemical Analysis

Analyzing chemical constituents of:

a. Vernonia amygdalina leaves
b. Jatropha curcas leaves
c. Crude oil samples
d. Soil samples

For phytoremediation potential assessment.

Materials Used

a. Muffle furnace, crucibles, evaporating dish
b. Kjeldahl digesting set, flask (100mL)
c. Soxhlet extraction set, beakers (250mL)
d. Hot-air drying oven

For analyzing chemical composition of plant leaves, crude oil, and soil samples
The pH of the soil sample was determined using a pH meter.
pH Determination

a. 5g soil + 10mL distilled water
b. pH meter probe inserted, reading taken

Soil pH assessed for understanding chemical properties and bioremediation potential
TPH analysis conducted using Agilent 7697A Headspace Autosampler, Agilent 7890A Gas Chromatograph (GC), and 5975C Mass Spectrometer (MS).

TPH Determination

a. Extraction with tetra chloromethane
b. Analysis using GC-MS (Agilent equipment)

Assessing crude oil contamination level in soil.

Sample Preparation

The samples were prepared for analysis as follows:

a. Sample Transfer: A portion of the sample was transferred to a tared 20 mL headspace vial using forceps.
b. Weighing: The sample was weighed, and the mass was recorded.
c. Sealing: The vial was sealed with a High-Performance septum.
d. Sample Mass: 10g of sample was used for all analyses, including:
    1. Jatropha Curcas
    2. Vernonia Amygdalina
    3. Bonny Light crude oil

This preparation ensures accurate and consistent analysis of the samples using the GC/MS Headspace technique.

Analytical Conditions

The analysis was performed using:

Instrument

a. Agilent 7697A Headspace Autosampler

b. Agilent 7890A Gas Chromatograph (GC)
c. 5975C Mass Spectrometer (MS) operated in negative EI mode

Conditions

a. Carrier Gas: Helium
b. Headspace Oven Temperature: 250°C
c. Equilibration Time: 60 minutes
d. MS Scan Range: 35-550 amu (atomic mass units)

Notes

1. The lower MS scan range was set to 35 amu to avoid interferences from atmospheric air (e.g., N_2, O_2, moisture).
2. Components with m/z <35 (e.g., methanol, formaldehyde) are not detected due to the scan range limitations.These conditions enable the detection and identification of hydrocarbon constituents in the samples, providing valuable information for the analysis.

Heavy Metals: Potential Risks and Behavior in Soil.

Heavy metals pose significant risks to the environment and human health due to their potential for bioaccumulation and biomagnification in the food chain. The most common heavy metals found at contaminated sites, in order of abundance, are:

a. Lead (Pb)
b. Chromium (Cr)
c. Arsenic (As)
d. Zinc (Zn)
e. Cadmium (Cd)
f. Copper (Cu)
g. Mercury (Hg)

Risks Associated with Heavy Metals

a. Decreased crop production: Heavy metals can accumulate in crops, reducing yields and affecting food quality.
b. Bioaccumulation and biomagnification: Heavy metals can accumulate in organisms and magnify up the food chain, posing risks to human health.
c. Watercontamination: Heavy metals can contaminate surface and groundwater, affecting human consumption and ecosystem health.

Behavior of Heavy Metals in Soil

Once in the soil, heavy metals undergo various reactions that affect their bioavailability, mobility, and toxicity. These reactions include:

a. Mineral precipitation and dissolution
b. Ion exchange, adsorption, and desorption
c. Aqueous complexation
d. Biological immobilization and mobilization
e. Plant uptake.

Bioremediation Experiment.

The bioremediation experiment utilized:

Materials

a. Reactors: 30 plastic bottles cut open
b. Soil samples: Contaminated soil samples
c. Crude oil: Bonny Light crude oil

d. Plant extracts:
   1. Vernonia Amygdalina leaf extract
   2. Jatropha Curcas leaf extract.

Pre-Analysis Test

The pre-analysis test includes:

Tests Conducted

a. Soil Analysis: Sandy Soil, samples were tested by BGI Laboratory Limited to establish a baseline for the bioremediation process.
b. Crude Oil Analysis: Gas Chromatography was used to determine the properties of the Bonny Light crude oil.
c. Microbial and Phytochemical Screening: Leaf extracts of Vernonia Amygdalina and Jatropha Curcas were analyzed to:
   1. Identify microorganisms present in the leaves that can aid in the bioremediation process.
   2. Determine the phytochemical composition of the leaf extracts.These tests provide essential baseline data for evaluating the effectiveness of the bioremediation process and understanding the potential of the plant extracts in enhancing biodegradation.

Bioremediation Experiment Procedures.

The experiment involved the following steps:

Soil Contamination and Treatment

a. Crude Oil Introduction: 50mL of Bonny Light crude oil was added to the soil samples to simulate pollution.
b. Mixing and Stirring: The soil samples were thoroughly mixed to achieve uniform concentration of crude oil.
c. Extract Application: Vernonia Amygdalina and Jatropha Curcas extracts were added to the reactors in varying amounts (10g-40g).

Experimental Design

1. Remediation Effect: The effects of the leaf extracts on the soil were monitored over 30 days.
2. Application Methods: Three different application methods were used:
   1. Sun-dried leaves
   2. Room-dried leaves
   3. Wet blended leaves
3. Moisture Maintenance: 5mL of water was added to the reactors every 5 days to maintain moist conditions.

Monitoring and Measurements

a. Parameters Measured: pH, hydrocarbon content, metal concentrations, and microbial activity were measured every 5 days.
b. Duration: Measurements were taken from day 0 to day 30.

Intermediate Testing

4. The intermediate testing involves analysing the soil-crude mixture after two weeks of experimentation. The tests conducted during this phase include:

Tests Conducted

a. pH Measurement: To monitor changes in soil pH.
b. Hydroarbon Content Analysis: To assess the reduction in hydrocarbon levels.

c. Metal Concentration Analysis: To determine changes in metal concentrations.
d. Microbial Activity Measurement: To evaluate the impact of leaf extracts on microbial populations.

Purpose

The intermediate testing aims to:

1. Monitor Bioremediation Progress: Track the effectiveness of the leaf extracts in remediating the contaminated soil.
2. Identify Trends: Determine which variables are decreasing or remaining constant over time.
3. Inform Future Decisions: Use the data collected to adjust the experimental approach or optimize the bioremediation process.

Data Collection

Data on metal concentrations (Pb, Zn, Cr), hydrocarbon contents, pH levels, application methods (sun-dried, room-dried, wet blended), and leaf extract weights (10g-40g) will inform phytoremediation strategies for crude oil polluted sandy soil, optimizing Jatropha curcas and Vernonia amygdalina leaf extract applications.

Model Generation

The goal of model generation is to create a framework that explains the bioremediation process and facilitates interpretation of results. This involves:

Objectives

a. Data Analysis: Analyze collected data to identify patterns and trends.
b. Model Development: Develop a model that describes the relationships between variables (leaf extract weight, soil type, pH, hydrocarbon content).
c. Result Interpretation: Use the model to interpret results.

RESULTS AND DISCUSSIONS

Bio-Remedial Analysis

The bioremediation process was monitored at regular intervals to assess the effectiveness of Vernonia Amygdalina and Jatropha Curcas leaf extracts in remediating crude oil-polluted soil. The analysis considered the following factors:

a. Leaf Extract Preparations:
   Room-dried leaf extract
   Sun-dried leaf extract
   Wet-blended leaf extract (blended with contaminated soil)
b. Soil Types: Sandy Soil.
c. Plant Extracts:
   Vernonia Amygdalina
   Jatropha Curcas
d. Time:
   Remediation progress was monitored over time to assess the effectiveness of the treatment.

Sandy Soil Bioremediation Analysis

The study investigated bioremediation in sandy-loamy soil contaminated with Bonny Light crude oil, replicating conditions found in Ogoni land. The analysis measured changes in pH, hydrocarbon content, and metal concentrations before and after crude oil addition.

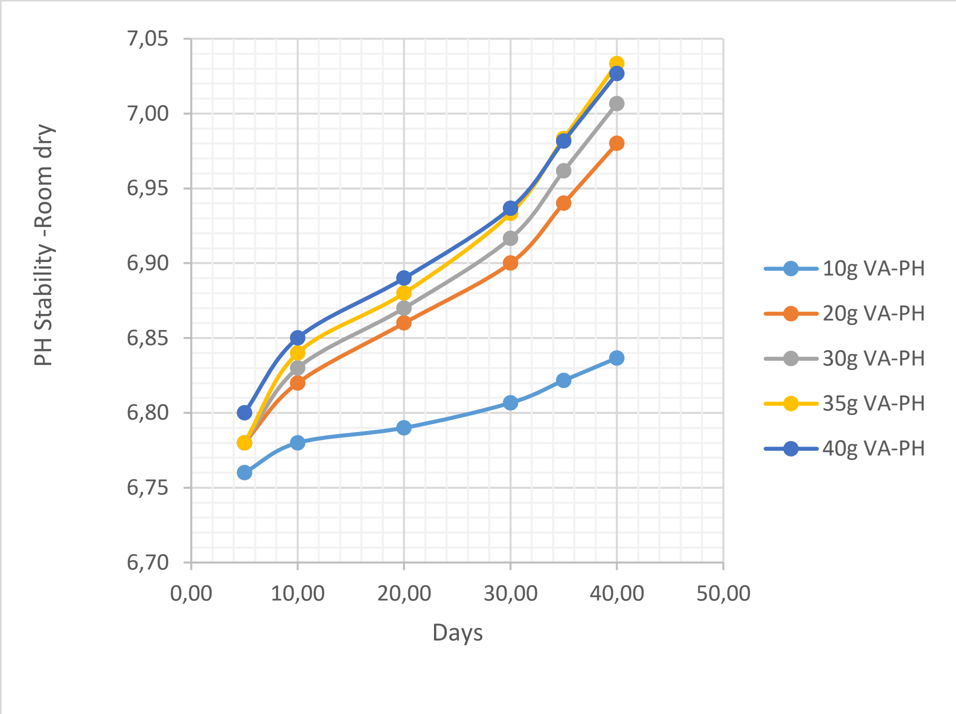

Fig 2 presents the initial and final conditions of the response factors, documenting the impact of crude oil contamination on the soil.

Table 1 Initial and Final readings of the Response factors for loamy soil

PH and HC readings for samples before contaminant					
Initial Content sample	PH	HC	Pb (ug/ml)	Zn (ug/ml)	Cr(ug/ml)
Sandy Soil, SSi	6.76	2.59	0.018	0.022	0.015
PH and HC readings for samples after contaminant					
Final Content sample	PH	HC	Pb (ug/ml)	Zn (ug/ml)	Cr(ug/ml)
Sandy Soil, SSf	6.75	4.67	1.22	0.923	1.103

pH Analysis

The study examined pH stability in contaminated soil by analyzing changes in pH levels as varying masses of Vernonia Amygdalina and Jatropha Curcas leaf extracts were added. The analysis considered different preparation methods, mass additions, and experimental durations to assess their impact on soil pH.

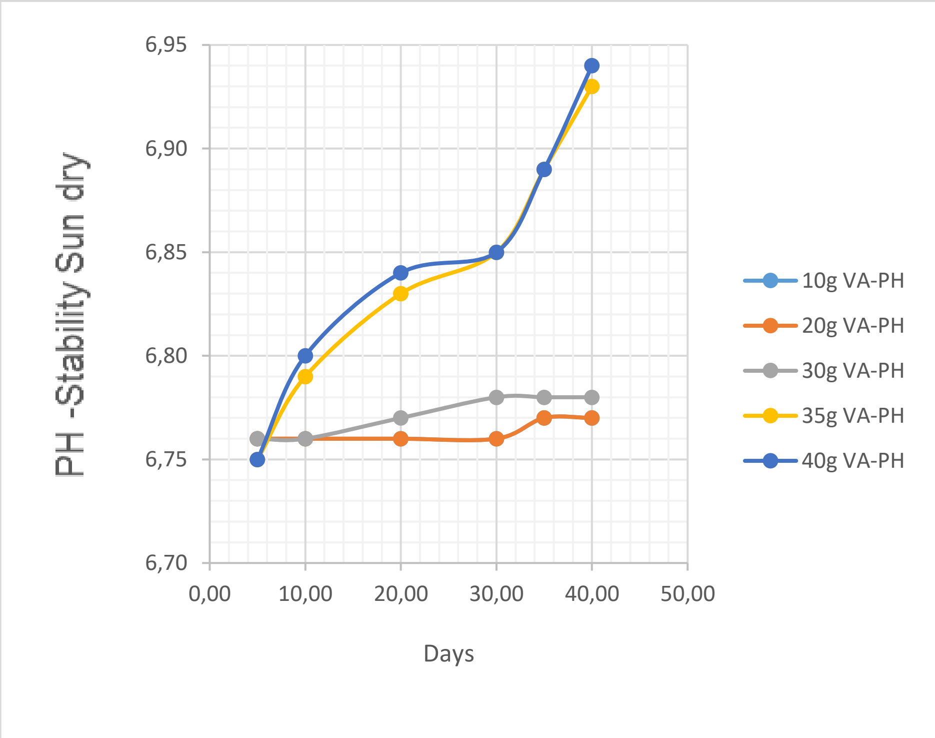

Fig 3: pH Behavioural Characteristics in Sandy Soil

The figure illustrates the pH stability and instability in sandy soil treated with Vernonia Amygdalina and Jatropha Curcas extracts using different application methods. The results show that only the sun-dried extracts at 10g and 20g maintained a stable pH, while other treatments increased the pH towards neutral due to metal reduction in the soil. This pH shift indicates the potential of these plant extracts for effective metal remediation in contaminated sandy soils

Hydrocarbon (HC) Analysis.

Vernonia Amygdalina is roughly twice as effective as Jatropha Curcas in remediating hydrocarbons in sandy soil.

Vernonia Amygdalina: 1.80 µg/mL

Jatropha Curcas: 0.90 µg/mL

(40g extract, 40 days**)**

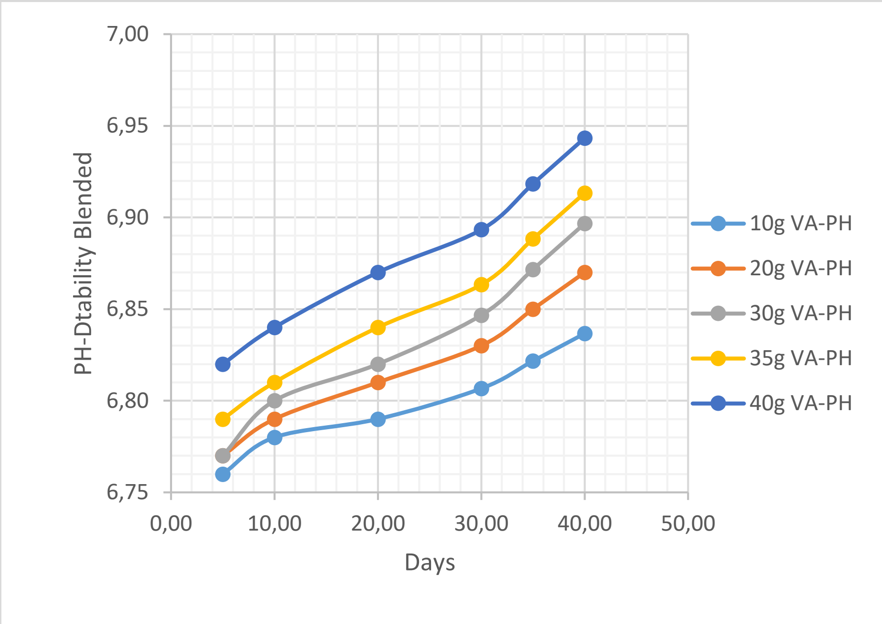

Fig 4: Hydrocarbon Content Remediation

The figure compares the effectiveness of Vernonia Amygdalina and Jatropha Curcas extracts in remediating hydrocarbon content in sandy soil.

Comparison of Preparation Methods

Wet-blended extracts of Vernonia Amygdalina (2.40 µg/mL) and Jatropha Curcas (2.11 µg/mL) showed highest hydrocarbon remediation potential in sandy soil.

Wet blending proved most effective vs. room-drying and sun-drying methods..

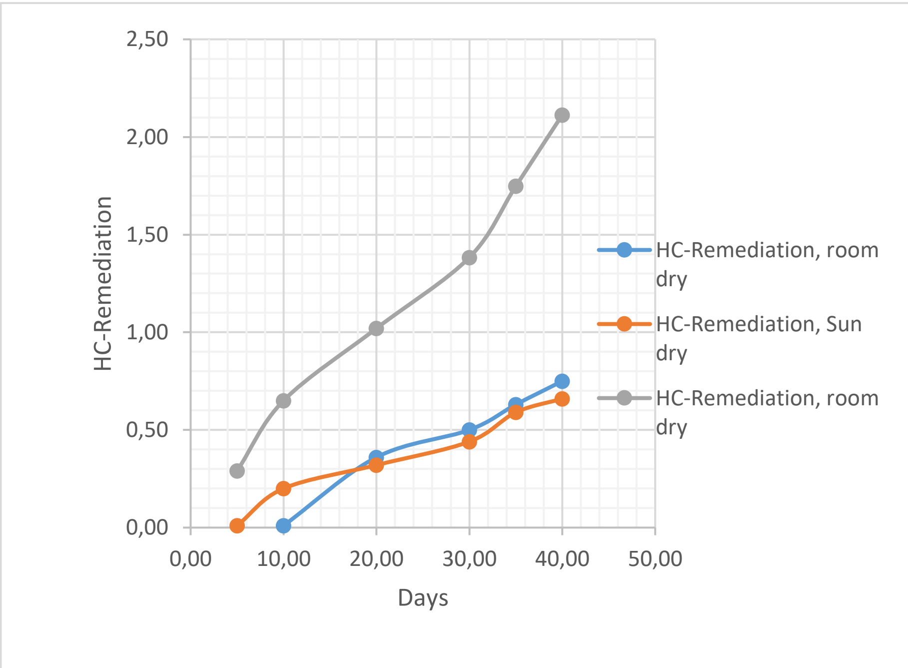

Fig 5: Hydrocarbon Content Remediation Method Comparison

The results demonstrate the potential of Vernonia Amygdalina and Jatropha Curcas extracts, particularly wet-blended preparations, for effective phytoremediation of crude oil polluted sandy soil.

Metal Analysis

Vernonia Amygdalina and Jatropha Curcas extracts reduced metal concentrations alongside hydrocarbons. Metal analysis shows pH shift towards neutral, indicating potential for simultaneous remediation of hydrocarbon and metal contamination in sandy soil

Pb Remediation Response in Sandy Soil

Vernonia Amygdalina and Jatropha Curcas extracts reduced Pb concentrations in sandy soil. Plant extracts show potential for Pb remediation alongside hydrocarbon removal in crude oil polluted soil.

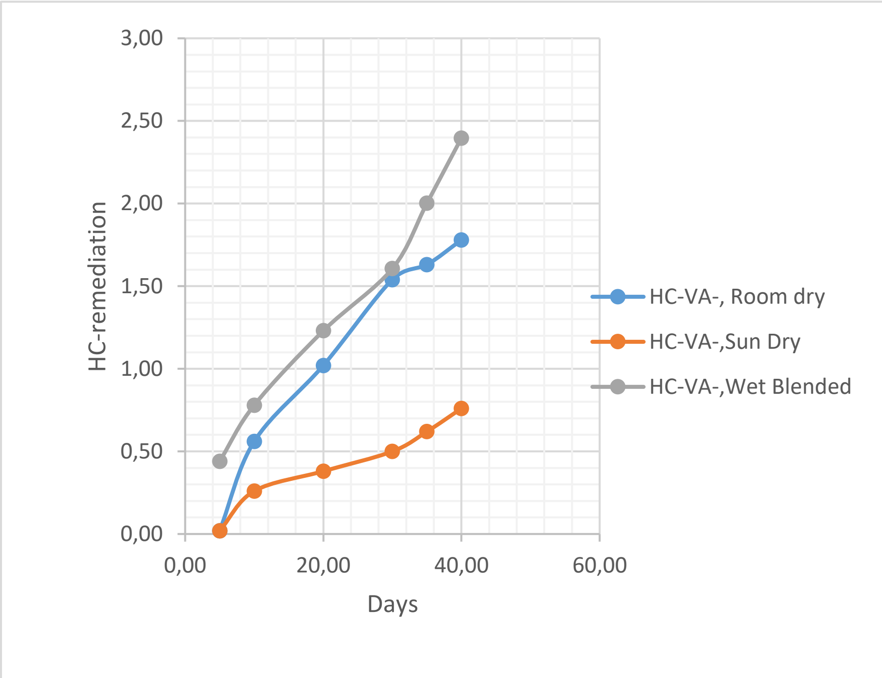

Fig 6: Pb Remediation Method Comparison

Wet blending enhances Pb remediation potential of Vernonia Amygdalina (0.99 µg/mL) and Jatropha Curcas (0.72 µg/mL) in crude oil polluted sandy soil

Zn Remediation Response

The study found that Zn remediation in sandy- soil was achieved using Jatropha Curcas and Vernonia Amygdalina extracts. Key findings include:

1. For Jatropha Curcas, the wet-blended extract showed a Zn remediation value of approximately 0.51 µg/mL, while room-dried and sun-dried extracts gave similar but lower results.
2. For Vernonia Amygdalina, the Zn remediation values were:
   Room-dried: 0.31 µg/mL
   Sun-dried: 0.52 µg/mL
   Wet-blended: 0.71 µg/mL
3. Overall, the wet-blended extract of Vernonia Amygdalina showed the best Zn remediation effect.

These results suggest that the wet-blended method enhances the Zn remediation potential of these plant extracts, with Vernonia Amygdalina showing promising results.

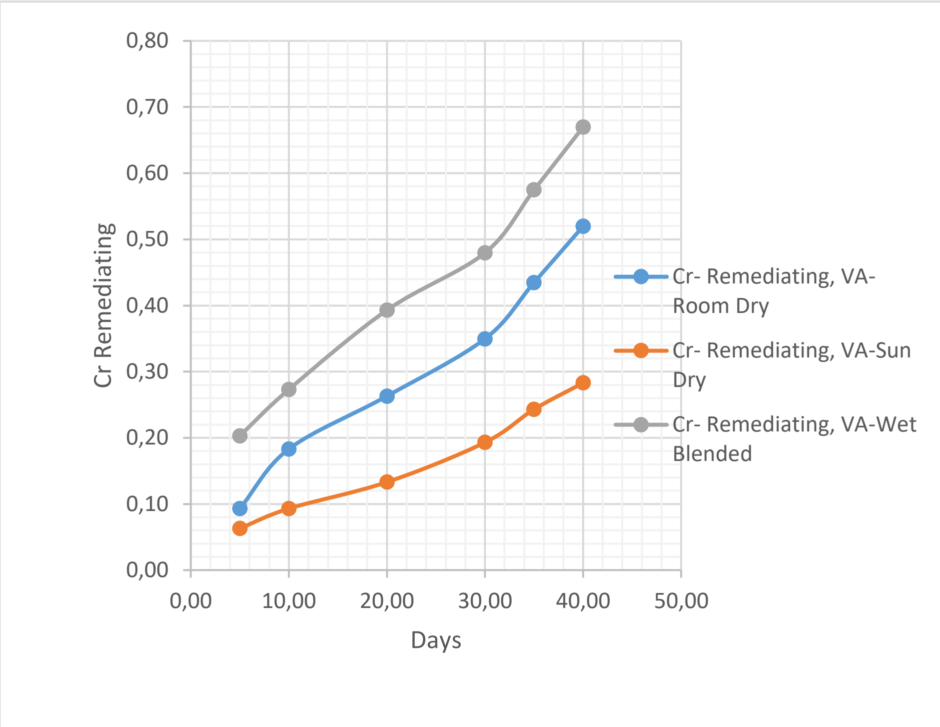

Fig 7: Zn Remediation Method Comparison

The figure compares the effectiveness of different preparation methods (room dry, sun dry, and wet blended) using Jatropha Curcas and Vernonia Amygdalina for Zn remediation in sandy soil. The results highlight the potential of these plant extracts and preparation methods for effective Zn removal, with the wet-blended method showing promising results, particularly for Vernonia Amygdalina.

Cr Remediation Response

The study found that the wet-blended method exhibited higher Cr remediation potential compared to other preparation methods. Both Jatropha Curcas and Vernonia Amygdalina showed similar Cr remediation values, with:

a. Jatropha Curcas: 0.67 µg/Ml
b. Vernonia Amygdalina: 0.68 µg/mL

These results suggest that the wet-blended method enhances the Cr remediation potential of both plant extracts.

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots + \beta_k x_{ik} + u_i \qquad \text{for } i = 1, \ldots, n.$$

In matrix form, we can rewrite this model as

$$\begin{bmatrix} y_1 \\ y_2 \\ \\ y_n \end{bmatrix} = \begin{bmatrix} 1 & x_{11} & x_{12} & \cdots & x_{1k} \\ 1 & x_{21} & x_{22} & \cdots & x_{2k} \\ \\ 1 & x_{n1} & x_{n2} & \cdots & x_{nk} \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \\ \beta_k \end{bmatrix} + \begin{bmatrix} u_1 \\ u_2 \\ \\ u_n \end{bmatrix}$$

n x 1   n x (k+1)   (k+1) x 1   n x 1

$$Y = X\beta + u$$

We want to estimate β.

Fig 8 Cr Remediation Method Comparison

The figure compares the effectiveness of different preparation methods using Jatropha Curcas and Vernonia Amygdalina for Cr remediation in sandy soil. The results show that the wet-blended method has the highest Cr remediation potential for both plant extracts, with Vernonia Amygdalina and Jatropha Curcas achieving remediation values of 0.68 μg/mL and 0.67 μg/mL, respectively.

Model Prediction Analysis

To further understand the remediation process, a predictive model was developed using the data generated from the wet-blended method, which showed promising results. The model illustrate the individual remedial activity of Vernonia Amygdalina and Jatropha Curcas extracts in soil, with measurable responses including metal and hydrocarbon content reduction. Independent factors considered was include:

a. Mass of plant extracts (Vernonia Amygdalina and Jatropha Curcas)
b. Remediation time (days)

A multiple regression analysis will be performed using the least squares method, facilitated by Minitab software, to develop a predictive model that can estimate remediation outcomes based on these factors

Sandy Soil Modeling Approach

To develop a predictive model for sandy soil, we'll use the least squares method to identify the relationships between key factors (such as time, mass, and pH) and heavy metal behavior, enabling accurate predictions and soil dynamics.

Jatropha Curcas Modelling

Regression Analysis: *HC versus Time, Mass, PH*

The regression equation is *HC = - 126 - 0.0207 Time - 0.0157 Mass + 18.6 PH*

Predictor	Coef	SE Coef	T	P
Constant	-125.51	38.99	-3.22	0.003
Time	0.02073	-0.01316	-1.58	0.127
Mass	-0.01570	0.01327	-1.18	0.247
PH	18.619	5.812	3.20	0.004

S = *0.328763 R-Sq = 62.3% R-Sq(adj) = 57.9%*

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	4.6396	1.5465	14.31	0.000
Residual Error	26	2.8102	0.1081		
Total	29	7.4498			

Model Analysis

The regression model for hydrocarbon (HC) concentration using Jatropha Curcas is:

HC = -126 - 0.0207(Time) - 0.0157(Mass) + 18.6(pH)

Key Observations:

a. pH: Has a significant positive impact on HC concentration, with a coefficient of 18.6, indicating a strong relationship.
b. Time and Mass: Have non-significant negative effects on HC concentration, suggesting a potential inverse relationship, but the effects are not statistically significant.
c. Model Fit: The model explains 62.3% of the variation in HC concentration, indicating a moderate fit.

Implications:

The model suggests that pH is a critical factor in determining HC concentration, and increasing pH levels may lead to higher HC concentrations. However, the effects of Time and Mass are not significant, and further investigation may be needed to understand their relationships with HC concentration

b. Regression Analysis: *Pb versus Time, Mass, PH*

The regression equation is *Pb = - 37.7 - 0.00290 Time + 0.00340 Mass + 5.56 PH*

Predictor	Coef	SE Coef	T	P
Constant	-37.681	6.988	-5.39	0.000
Time	-0.002902	0.002358	-1.23	0.229
Mass	0.003397	0.002378	1.43	0.165
PH	5.562	1.042	5.34	0.000

S = *0.0589214 R-Sq = 93.3% R-Sq(adj) = 92.5%*

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	1.25980	0.41993	120.96	0.000
Residual Error	26	0.09026	0.00347		
Total	29	1.35007			

Regression Analysis.

The regression model for lead (Pb) concentration reveals:

a. Significant Positive Relationship:
   pH has a highly significant positive effect on Pb concentration (p = 0.000).
b. Non-Significant Relationships:
   Time has a non-significant negative effect on Pb concentration (p = 0.229).
   Mass has a non-significant positive effect on Pb concentration (p = 0.165).
c. Excellent Model Fit:
   The model explains 93.3% of the variation in Pb concentration (R-Sq = 93.3%).
   The adjusted R-squared value is 92.5%, indicating an excellent model fit.

These findings suggest that pH is a crucial factor in determining Pb concentration, and the model can accurately predict Pb levels based on pH values

Regression Analysis: *Zn versus Time, Mass, PH*

The regression equation is *Zn = - 23.3 - 0.00135 Time + 0.00122 Mass + 3.45 PH*

Predictor	Coef	SE Coef	T	P
Constant	-23.321	1.407	-16.57	0.000
Time	-0.0013451	0.0004749	-2.83	0.009
Mass	0.0012178	0.0004789	2.54	0.017
PH	3.4524	0.2098	16.46	0.000

S = *0.0118667 R-Sq = 99.2% R-Sq(adj) = 99.1%*

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.45649	0.15216	1080.55	0.000
Residual Error	26	0.00366	0.00014		
Total	29	0.46015			

Regression Analysis.

The regression model for zinc (Zn) concentration reveals:

Significant Relationships:

1. pH has a highly significant positive effect on Zn concentration (p = 0.000).
2. Time has a significant negative effect on Zn concentration (p = 0.009).
3. Mass has a significant positive effect on Zn concentration (p = 0.017).

Excellent Model Fit:

a. The model explains 99.2% of the variation in Zn concentration (R-Sq = 99.2%).
b. The adjusted R-squared value is 99.1%, indicating an excellent model fit.

These findings suggest that pH, Time, and Mass are all important factors in determining Zn concentration, and the model can accurately predict Zn levels based on these factors.

Regression Analysis: Cr versus Time, Mass, PH

The regression equation is *Cr = - 18.6 + 0.00396 Time + 0.00195 Mass + 2.76 PH*

Predictor	Coef	SE Coef	T	P
Constant	-18.648	1.941	-9.61	0.000
Time	0.0039635	0.0006550	6.05	0.000
Mass	0.0019489	0.0006605	2.95	0.007
PH	2.7577	0.2893	9.53	0.000

S = *0.0163666 R-Sq = 99.0% R-Sq(adj) = 98.9%*

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.70052	0.23351	871.73	0.000
Residual Error	26	0.00696	0.00027		
Total	29	0.70749			

Chromium (Cr) Concentration Model

The model predicts Cr concentration based on Time, Mass, and pH. It shows that:

a. Increasing Time slightly increases Cr concentration.
b. Increasing Mass slightly increases Cr concentration.
c. Increasing pH significantly increases Cr concentration.

The model is very accurate, explaining 99% of the variation in Cr concentration.

Vernonia Amygdalina Modelling

a. Regression Analysis: HC_1 versus Time_1, Mass_1, PH_1

The regression equation is

HC_1 = - 145 - 0.0395 Time_1 - 0.0217 Mass_1 + 21.5 PH_1

Predictor	Coef	SE Coef	T	P
Constant	-144.86	22.10	-6.55	0.000
Time_1	-0.03950	0.01004	-3.93	0.001
Mass _1	-0.021669	0.008885	-2.44	0.022
PH_1	21.498	3.297	6.52	0.000

S = *0.208392 R-Sq = 87.5% R-Sq(adj) = 86.1%*

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	7.9029	2.6343	60.66	0.000
Residual Error	26	1.1291	0.0434		
Total	29	9.0320			

Hydrocarbon (HC) Concentration Model

The model predicts HC concentration using Vernonia Amygdalina. It shows that:

1. Increasing pH significantly increases HC concentration.
2. Increasing Time decreases HC concentration.
3. Increasing Mass decreases HC concentration.

The model is quite accurate, explaining 87.5% of the variation in HC concentration.

Regression Analysis: Pb_1 versus Time_1, Mass_1, PH_1

The regression equation is

Pb_1 = - 23.7 + 0.00058 Time_1 + 0.0130 Mass_1 + 3.48 PH_1

Predictor	Coef	SE Coef	T	P
Constant	-23.74	10.01	-2.37	0.025
Time_1	0.000577	0.004546	0.13	0.900
Mass _1	0.012987	0.004024	3.23	0.003
PH_1	3.484	1.493	2.33	0.028

S = 0.0943675 R-Sq = 90.6% R-Sq(adj) = 89.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	2.23640	0.74547	83.71	0.000
Residual Error	26	0.23154	0.00891		
Total	29	2.46794			

Regression Analysis

The regression model for lead (Pb) concentration reveals:

a. Significant Relationships:
   Mass has a significant positive effect on Pb concentration (p = 0.003).
   pH has a significant positive effect on Pb concentration (p = 0.028).
b. Non-Significant Relationship:
   Time has a non-significant effect on Pb concentration (p = 0.900).
c. Good Model Fit:
   The model explains 90.6% of the variation in Pb concentration (R-Sq = 90.6%).
   The adjusted R-squared value is 89.5%, indicating a good model fit.

These findings suggest that Mass and pH are important factors in determining Pb concentration.

Regression Analysis: Zn_1 versus Time_1, Mass_1, PH_1

The regression equation is

Zn_1 = - 4.66 + 0.00585 Time_1 + 0.0124 Mass_1 + 0.669 PH_1

Predictor	Coef	SE Coef	T	P
Constant	-4.662	3.611	-1.29	0.208
Time_1	0.005848	0.001641	3.56	0.001
Mass _1	0.012441	0.001452	8.57	0.000
PH_1	0.6695	0.5388	1.24	0.225

S = 0.0340525 R-Sq = 97.1% R-Sq(adj) = 96.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.99199	0.33066	285.16	0.000
Residual Error	26	0.03015	0.00116		
Total	29	1.02214			

Zinc (Zn) Concentration Model

The model predicts Zn concentration. It shows that:

1. Increasing Time increases Zn concentration.
2. Increasing Mass significantly increases Zn concentration.
3. pH has a minimal effect on Zn concentration.

The model is very accurate, explaining 97.1% of the variation in Zn concentration.

Regression Analysis: *Cr versus Time_1, Mass_1, PH_1*

The regression equation is

Cr = - 16.8 + 0.00258 Time_1 + 0.00153 Mass_1 + 2.48 PH_1

Predictor	Coef	SE Coef	T	P
Constant	-16.768	1.707	-9.82	0.000
Time_1	0.0025843	0.0007755	3.33	0.003
Mass _1	0.0015306	0.0006863	2.23	0.035
PH_1	2.4798	0.2547	9.74	0.000

S = 0.0160963   R-Sq = 99.0%   R-Sq(adj) = 98.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.70075	0.23358	901.55	0.000
Residual Error	26	0.00674	0.00026		
Total	29	0.70749			

Regression Analysis

The regression model for chromium (Cr) concentration reveals:

1. Significant Relationships:
   Time has a significant positive effect on Cr concentration (p = 0.003).
   Mass has a significant positive effect on Cr concentration (p = 0.035).
   pH has a highly significant positive effect on Cr concentration (p = 0.000).
2. Excellent Model Fit:
   The model explains 99.0% of the variation in Cr concentration (R-Sq = 99.0%).
   The adjusted R-squared value is 98.9%, indicating an excellent model fit.

These findings suggest that Time, Mass, and pH are all important factors in determining Cr concentration, and the model can accurately predict Cr levels based on these factors

From the different models developed, the major concern is the p-value which is the probability value and the r^2 value which is the co-efficient of determination. Statistically, a model is accepted and is said to be significantly accepted if the overall p-value of the model is less than 0.05. the r^2 shows the relationship between the variables, the higher the value closer to 100% the better.

Research Summary

This study demonstrates the efficacy of Jatropha curcas and Vernonia amygdalina leaf extracts in bioremediating hydrocarbon and metal-contaminated sandy soil. Key findings:

a. > 50% reduction in contaminants (40 days)
b. Effective removal of Pb, Zn, and hydrocarbons
c. Regression models ($R^2 > 0.875$) support eco-friendly phytoremediation potentia.

CONCLUSION

This study demonstrates the efficacy of Jatropha curcas and Vernonia amygdalina leaf extracts in phytoremediating crude oil-polluted sandy soil. Wet-blended extracts (40g, 40 days) showed significant removal of:

a. Hydrocarbons: 2.40 μg/mL (Vernonia amygdalina), 2.11 μg/mL (Jatropha curcas)
b. Lead (Pb): 0.99 μg/mL (Vernonia amygdalina), 0.72 μg/mL (Jatropha curcas)
c. Zinc (Zn): 0.71 μg/mL (Vernonia amygdalina), 0.51 μg/mL (Jatropha curcas)

Regression models explain 87.5-99.0% of variation, highlighting the potential of these plant extracts as eco-friendly phytoremediation agents for crude oil-polluted soils.

Contribution to knowledge

In this thesis, I have been able to establish that the Jatropha curcas and Vernonia Amygdalina can be used for bio-remediation and have developed regression models to know and predict the extent of materials and time required to perform remediation activities in the three types of soil for the required contaminants.

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