



## Comparative Efficacy of Plant Leaves in Remediation of Crude Oil Polluted Silty Soil

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### ABSTRACT

This study compared the efficacy of *Jatropha curcas* and *Vernonia amygdalina* leaf extracts in bioremediating crude oil-polluted silty soil through composite bioaugmentation. Wet-blended extracts (with *P. aeruginosa*, *S. aureus*, *E. coli*) achieved >50% contaminant reduction in 40 days. Predictive models (Minitab) explained remediation outcomes, highlighting the potential of these plant extracts as eco-friendly agents for silty soil restoration in Ogoniland, Niger Delta, Nigeria. The study investigated the comparative efficacy of *Jatropha curcas* and *Vernonia amygdalina* leaf extracts in bioremediating crude oil-polluted soils through composite bioaugmentation. The leaf extracts, harboring *P. aeruginosa*, *S. aureus*, and *E. coli*, were prepared via sun drying, room drying, and wet blending, and applied to contaminated sandy, loamy, and silt soils. Results showed that wet-blended extracts demonstrated superior remediation performance, achieving over 50% reduction in contaminant levels. Treatment with 10-40g of leaf extract over 40 days significantly reduced contaminants, with optimal results observed in sandy soils. Using Minitab software, predictive models were developed to estimate the impact of leaf mass, treatment time, and soil pH on the remediation of hydrocarbons, lead, zinc, and chromium. The models were statistically significant ( $p < 0.05$ ) with good explanatory power (high  $R^2$ ), indicating their potential for predicting remediation outcomes. These findings highlight the potential of *Jatropha curcas* and *Vernonia amygdalina* as effective bio-remediation agents for crude oil-polluted soils, offering a sustainable solution for environmental restoration.

#### Keywords:

Minitab software, Silty, *Jatropha curcas*, *Vernonia amygdalina*, Crude Oil.

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## INTRODUCTION

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 efficiency. Crude 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.

### **Statement of the Problem**

The Ogoniland region has been severely impacted by decades of oil exploration and spills, resulting in widespread soil contamination and environmental degradation. The introduction of foreign bodies like olefins, bituminous contents, heavy metals, and other sulphuric contents has rendered the soil infertile, posing a significant threat to the livelihoods of the Ogoni people and their future generations. If left unaddressed, the

contamination is likely to persist for centuries, exacerbating food insecurity, health risks, and economic instability in the region. The need for effective and sustainable remediation strategies is urgent, and phytoremediation, coupled with bioaugmentation, offers a promising approach. However, the efficacy of different plant species and microorganisms in remediating crude oil-polluted soils in Ogoniland remains unclear. This study aims to investigate the potential of plant leaves and bioaugmentation to restore soil fertility and promote sustainable agriculture in Ogoniland, ultimately contributing to the well-being of the local community.

### **Key Concerns**

- a. Soil contamination: Widespread pollution of soil due to oil spills
  - b. Environmental degradation: Long-term damage to ecosystems and biodiversity
  - c. Food insecurity: Impact on agricultural productivity and food availability
  - d. Health risks: Potential health hazards due to exposure to pollutants
  - e. Economic instability: Negative impact on local economies and livelihoods
- Problem statement.

### **Research Aim:**

The aim of this research is to compare and evaluate the effectiveness of composite bioaugmentation of plant leaves in remediation of crude oil polluted silty soil, with the goal of identifying optimal plant-microbe combinations for enhanced soil fertility restoration and sustainable agriculture.

### **Objectives:**

1. To evaluate the effectiveness of *Jatropha Curcas* and *Vernonia Amygdalina* leaf extracts in remediating crude oil-polluted silty soil.
2. To compare the remediation potential of *Jatropha Curcas* and *Vernonia Amygdalina* leaf extracts at different concentrations in silty soil.
3. To investigate the relationship between leaf extract concentration and remediation efficiency in silty soil.
4. To develop a statistical model that predicts the bio-remediation process using *Jatropha Curcas* and *Vernonia Amygdalina* leaf extracts in silty soil.

### **Significance of the Study**

Achieving the aim and objectives of this study will provide valuable insights into the effectiveness of *Jatropha Curcas* and *Vernonia Amygdalina* leaf extracts in remediating crude oil-polluted silty soil. The findings of this study will:

1. Inform optimization strategies: Provide institutions with data to optimize bio-remediation activities using plant leaves and microorganisms in silty soil.
2. Benefit affected communities: Offer hope to communities in Nigeria, such as those in the Niger Delta region, who are struggling with the impacts of oil pollution on their environment and livelihoods.
3. Advance knowledge: Contribute to the existing body of knowledge on bio-remediation, stimulating further research and development in this field, specifically in the context of silty soils.
4. Support sustainable development: Enhance understanding of sustainable approaches to environmental remediation, promoting eco-friendly solutions for polluted silty soil management.

### **Contribution to UN Sustainable Development Goals (SDGs)**

This research contributes to several UN SDGs:

- a. 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.
- b. 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.
- c. 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.
- d. 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.
- e. 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.
- f. SDG 15: Life on Land- By restoring ecosystems and promoting biodiversity, this research can help protect and conserve terrestrial ecosystems, promoting life on land.
- g. By contributing to these SDGs, this research can help promote sustainable development, reduce environmental degradation, and improve human well-being.

#### **Review of Previous Works in Bio-remediation**

Since the 1970s, bioaugmentation, or the addition of oil degrading microorganisms to supplement the indigenous populations, has been proposed as an alternate strategy for the bioremediation of oil contaminated environments. The rationale for this approach is that indigenous microbial populations may not be capable of degrading the wide range of potential substrates present in complex mixtures such as petroleum (Leahy and Colwell, 1990) or that they may be in a stressed state as a result of the recent exposure to the spill. Other conditions under which bioaugmentation may be considered are when the indigenous hydrocarbon-degrading population is low, the speed of decontamination is the primary factor, and when seeding may reduce the lag period to start the bioremediation process (Forsyth et al., 1995). For this approach to be successful in the field, the seed microorganisms must be able to degrade most petroleum components, maintain genetic stability and viability during storage, survive in foreign and hostile environments, effectively compete with indigenous microorganisms, and move through the pores of the sediment to the contaminants (Atlas, 1977; Goldstein et al., 1985). Methods involving the addition of selected oil-degrading microorganisms into spilled oil have been patented and marketed since early 1970s (Azarowick, 1973; Linn, 1971; and Mohan et al., 1975). However, before the Exxon Valdez spill in 1989, little information on the performance of commercial bioaugmentation products was available in the peer-reviewed literature. Atlas and Bartha (1973) conducted one of the first laboratory tests on the effectiveness of commercial mixed bacterial cultures. Two commercial petroleum-degrading bacterial inocula, Ekolo-Gest (also marketed as Petrobac, National Chem. Corp.) and DBC bacteria (Gerald Bauer Corp.), were tested using shake flasks to compare the degradation of Sweden crude oil. The study found that none of the commercial mixtures was superior to the indigenous microorganisms in coastal marine waters.

One of first field trials on oil bioremediation using a microbial product in a marine environment was reported by Lee and Levy (1987). The study involved seeding a mixed

culture of marine oil-degrading bacteria (strains of *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, and *Bacillus subtilis* grown on bran) in a Scotian Shelf Condensate (SSC) contaminated sandy beach.

The extent of biodegradation was measured by the decline in the n-C17/pristane ratio in this study. The results showed that the n C17/pristane ratio in the seeded plots did decrease slightly. However, due to high inter-and intra-plot variability, no significant difference in the rate of oil loss was observed among the treatments. This study also observed that the number of oil degrading bacteria did not increase until 10 to 15 days after the addition of oil. However, the addition of the microbial product did not reduce this lag period, suggesting that the toxic volatile components in the oil, which evaporated mostly during the first week, was the main cause of the lag period.

Since the application of nutrient amendments for the clean-up of the Exxon Valdez spill in 1989, bioremediation has received increased attention, and several field tests and applications of bio augmentation have been reported. Venosa et al. (1992) conducted a field test in Prince William Sound following the Exxon Valdez spill to investigate the effectiveness of two commercial microbial products vis-à-vis natural attenuation and nutrient addition alone. These products were selected based on a previous laboratory study (Venosa et al., 1991). This field trial failed to demonstrate enhanced oil biodegradation by these products. No bio stimulation occurred in the nutrient control plots either. There were no significant differences between any of the treatment and control plots during the 27-day trial period. However, the site where the project took place (Disk Island) was characterized as having highly weathered (degraded) oil and very calm waters, so dissolved oxygen may have been limiting, thus precluding effective biodegradation by any means.

One approach in overcoming the competition problem was proposed by Rosenberg et al. (1992). They developed a product that combined a polymerized urea formaldehyde fertilizer, which they called F-1, with a selected oil-degrading culture capable of using this fertilizer as a nitrogen source. Thus, the culture had a selective advantage over the indigenous population unable to utilize F-1 as nutrient source. A field trial conducted at an Israeli beach showed that this approach seemed to be successful in enhancing oil biodegradation. However, conclusions were confounded by the lack of adequate controls in the study (Swannell et al., 1996; Venosa, 1998). To evaluate the effectiveness of two commercial bio augmentation products in an estuarine environment, a field trial was carried out in a Texas coastal wetland by a research group from Texas A&M University (Simon et al., 1999; Townsend et al., 1999). The two products were selected based on a previous laboratory efficacy test, in which four out of twelve products showed an enhancement of oil biodegradation with significantly higher degradation rates of alkanes and aromatics when compared to a nutrient control (Aldrett et al., 1997). The 21- plot site, named San Jacinto Wetland Research Facility (SJWRF) has been used for a series of studies on oil spills and their countermeasures. In this study, four treatment strategies were examined: an oiled control, biostimulation with inorganic nutrient addition (diammonium phosphate), and commercial bioaugmentation with 2 different products. Arabian medium crude oil was selected in this test and the 21 plots each measuring 5 x 5 m were arranged in a balanced, incomplete block experimental design. Oil constituents were determined using gas chromatography/mass spectrometry (GC/MS) and were normalized to 17 $\alpha$ (H), 21 $\beta$ (H)-hopane to reduce the effects of sample heterogeneity and physical losses. The results showed that the addition of microbial products could not significantly enhance oil

biodegradation rates. No differences were observed between treatments when comparing the first order biodegradation rate coefficients for the total target saturates, total target aromatics, and individual hydrocarbon target analytes. The authors also pointed out that one of the products (BP8) “did show consistently higher biodegradation rates, though the rates were not significantly different from the control.” Because this microbial product was applied with vendor supplied inorganic nutrients (Townsend et al., 1999), it is difficult to conclude whether the “consistently but insignificantly” higher rates resulted from the additions of the microbial components or the nutrient components. The fact that neither addition of bioaugmentation agents nor application of inorganic nutrients significantly enhanced oil biodegradation suggested that other factors, such as oxygen, could have been limiting oil degradation in that environment. Studies comparing the performance of bioaugmentation and biostimulation have suggested that nutrient addition alone had a greater effect on oil biodegradation than did the addition of microbial products when oxygen supply was not limited (Jobson et.al., 1974; Lee et.al., 1997; Venosa et.al., 1996). This is probably because the hydrocarbon-degrading population is rarely a limiting factor as compared to the nutrients since the size of the hydrocarbon degrading bacterial population usually increases rapidly in response to oil contamination. One of the first comprehensive field tests evaluating various bioremediation approaches to enhance oil biodegradation was carried out in a soil environment in northwest area of Canada in early 1970s (Jobson et.al., 1974). A randomized block design was used to examine the effects of four treatments (control, inorganic fertilizer application, addition of a microbial culture alone, and combined fertilizer and microbial culture addition) over a 308-day time period. The microbial culture was grown in the laboratory and consisted of several genera of oil-degrading bacteria (*Flavobacterium* and *Cytophoga* sp., *Pseudomonas* sp., *Xanthomonas* sp., *Alcaligenes* sp., and *Arthrobacter* sp.). The study showed that the nutrient application resulted in a significant stimulation of bacterial numbers and in the degradation rate of n-alkane components of the crude oil. The application of the microbial agent, however, resulted in only a slightly enhanced degradation rate of n-alkane components of chain lengths C20 to C25.

A field study conducted on a sandy beach in Delaware also showed that addition of a microbial inoculum did not enhance oil biodegradation more than addition of inorganic nutrients alone (Venosa et.al., 1996). A randomized block design was used in this study to assess the effects of three treatments: a no-nutrient control (natural attenuation), addition of water-soluble nutrients, and addition of water-soluble nutrients supplemented with a natural microbial inoculum from the site. No significant differences were observed between plots treated with nutrients alone and plots treated with nutrients and the indigenous inoculum, suggesting that supplementation of the natural population with indigenous cultures from the same site still did not result in further enhancement over simple nutrient addition on marine shorelines. The authors also indicated that this conclusion could be extended to include exogenous microbial inoculate or commercial microbial agents because “if indigenous cultures do not accelerate the degradation rates, organisms enriched from different environments, grown in the laboratory, and not acclimated to a particular climatic or geographic location should be even less able to compete with the natural population.”

Lee et.al., (1997) conducted a 129-day field trial to compare the effect of four treatments on biodegradation of weathered Venture Condensate on a sandy beach in Nova Scotia, Canada. The four treatments (control, inorganic nutrient addition, a commercial

bioremediation product, and addition of inorganic nutrients along with bioremediation product) as well as an unoiled control were replicated in a complete block design using 20 enclosures or plots. C2-chrysene was used as the normalizing biomarker due to the low concentration of hopane in the condensate.

PRP (PetrolRem, Inc.) was selected to be the representative commercial bioremediation agent in this study. This product is no longer listed in the current NCP Product Schedule. According to Lee et al. (1997), PRP contains mineral nutrients and non-pathogenic bacteria within spherical particles made from plant derived natural products (beeswax) and exhibits both bio-augmentation and bio-stimulation properties. The agricultural fertilizer used in this study was a mixture of granular forms of ammonium nitrate (N.P.K. 33-0-0) and triple super phosphate (N.P.K. 0-46-0).

The study showed that an average of 11.0% of the n-alkanes remained in the oiled control plots, and only 0.1% of the oil remained in the enclosures treated with inorganic nutrients alone; 5.4% of the alkanes were found in the plots treated with inorganic nutrients and PRP, and 25.3% remained in the plots treated with PRP alone. The results indicate that periodic addition of inorganic nutrients was the most effective strategy for enhancing oil degradation and that the full potential of the bioremediation product was limited by nutrient availability. This field trial demonstrated that adding the bioremediation product did not perform better in terms of enhancing alkane degradation than applying inorganic agricultural fertilizers alone.

Several other possible reasons for the failure of inocula in degrading contaminants in nature were summarized by Goldstein et al. (1985), which include: (1) the concentration of the contaminant may be too low to support the growth of the inoculated species, (2) the natural environment may contain substances inhibiting growth or activity of the inocula, (3) the growth rate of the inoculated species may be limited by predation such as protozoa, (4) the added species may use other substrates in nature rather than the targeted contaminants, and (5) the seeded microorganisms may be unable to move through the pores of the sediment to the contaminants.

A few field trials did claim success in demonstrating the effectiveness of oil bioaugmentation, such as using Alpha BioSea™ (Alpha Environmental, Inc.) to treat the Angolan Palanca crude oil spilled from Mega Borg off Texas coast (Mauro and Wynne, 1990; Swannell et al., 1996) and using TerraZyme™ (Oppenheimer Biotechnology) in enhancing biodegradation of a heavy oil spilled from Nakhodka in Japan (Tsutsumi et al., 2000). However, the success of these studies was based on either visual observation (i.e. the Mega Borg study) or digital photographic image analysis (i.e., the Nakhodka study). No comprehensive monitoring program was used to verify the oil was indeed removed through enhanced biodegradation. The two products basically contain the same bacterial cultures and nutrients (Hozumi et al., 2000). The observed visual effects might have been due to physical or chemical processes such as surfactant action associated with the products (Swannell et al., 1996) or sinking.

All these peer-reviewed journal articles show that even though the addition of microorganisms may be able to enhance oil biodegradation in the laboratory, the effectiveness of bio augmentation has not been convincingly demonstrated in the field. Actually, most field studies indicated that bio augmentation is not effective in enhancing oil biodegradation in inland, estuarine, and marine environments. It appears that in most environments, indigenous oil degrading microorganisms are more than sufficient to carry

out oil biodegradation if nutrient levels and other adverse environmental conditions do not limit them.

### **Environmental issues in the Niger Delta**

The delta covers 20,000 km<sup>2</sup> within wetlands of 70,000 km<sup>2</sup> formed primarily by sediment deposition. Home to 20 million people and 40 different ethnic groups, this floodplain makes up 7.5% of Nigeria's total land mass. It is the largest wetland and maintains the third-largest drainage basin in Africa. The Delta's environment can be broken down into four ecological zones: coastal barrier islands, mangrove swamp forests, freshwater swamps, and lowland rainforests.

This incredibly well-endowed ecosystem contains one of the highest concentrations of biodiversity on the planet, in addition to supporting abundant flora and fauna, arable terrain that can sustain a wide variety of crops, lumber or agricultural trees, and more species of freshwater fish than any ecosystem in West Africa. The region could experience a loss of 40% of its inhabitable terrain in the next thirty years as a result of extensive dam construction in the region. The carelessness of the oil industry has also precipitated this situation, which can perhaps be best encapsulated by a 1983 report issued by the NNPC, long before popular unrest surfaced.

Since then, there has been the slow poisoning of the waters of this country and the destruction of vegetation and agricultural land by oil spills which occur during petroleum operations. But since the inception of the oil industry in Nigeria, more than twenty-five years ago, there has been no concerned and effective effort on the part of the government, let alone the oil operators, to control environmental problems associated with the industry. [Bogumil, 2014].

Reports on the extent of the oil spills vary. The Department of Petroleum Resources estimated 1.89 million barrels of petroleum were spilled into the Niger Delta between 1976 and 1996 out of a total of 2.4 million barrels[Vidal, 2010] spilled in 4,835 incidents. [The Daily Independent,2010] (approximately 220 thousand cubic metres). A UNDP report states that there have been a total of 6,817 oil spills between 1976 and 2001, which account for a loss of three million barrels of oil, of which more than 70% was not recovered.[UNDP,2006] 69% of these spills occurred off-shore, a quarter was in swamps and 6% spilled on land.

The Nigerian National Petroleum Corporation places the quantity of petroleum jettisoned into the environment yearly at 2,300 cubic metres with an average of 300 individual spills annually.[Bronwen,2007] However, because this amount does not take into account "minor" spills, the World Bank argues that the true quantity of petroleum spilled into the environment could be as much as ten times the officially claimed amount. [Moffat and Linden, 2009] The largest individual spills include the blowout of a Texaco offshore station which in 1980 dumped an estimated 400,000 barrels (64,000 m<sup>3</sup>) of crude oil into the Gulf of Guinea and Royal Dutch Shell's Forcados Terminal tank failure which produced a spillage estimated at 580,000 barrels (92,000 m<sup>3</sup>).[Nwilo,2001] In 2010 Baird reported that between 9 million and 13 million barrels have been spilled in the Niger Delta since 1958.[Baird, 2010] One source even calculates that the total amount of petroleum in barrels spilled between 1960 and 1997 is upwards of 100 million barrels (16,000,000 m<sup>3</sup>).[Bronwen,2007].

### **Bioremediation technologies for crude oil contaminated sites**

Bioremediation is a technology that exploits the abilities of microorganisms and other natural habitat of the biosphere to improve environmental quality for all species, including man. The development of innovative bioremediation technology as a functional tool in clean-up of crude oil polluted environment has depended so much on the basic knowledge of the physiology and ecology of the natural bacterial populations found in such polluted sites. Many advances in biochemistry and molecular biology are now applied in various bioremediation efforts (Olson and Tsai, 1992; Bouwer, 1992). According to some investigators (Barbee, et.al., 1996; Ritter and Scarborough, 1995), bioremediation does not always result in complete mineralization of organic compounds. Many of these compounds are naturally transformed to metabolites of unknown persistence and toxicity. Therefore, some basic steps that may be necessary for a successful bioremediation project will include compliance analysis, site characterization, method selection / feasibility studies, remediation proper and end for project analysis (Bonaventura, *et al.*, 1995). Compliance analysis requires examination of the contaminated site in the light of the governing regulation and the action plan. Examination of the site will lead to its characterization and this is a very challenging and difficult aspect of a bioremediation efforts. Knowledge of soil parameters such as cation exchange capacity, relevant nutrient availability, acidity (soil pH), aeration or oxygen level, hydraulic properties etc. are paramount and this requires the assistance of specialists in these areas. The last stage of any bioremediation project should include bioassay of the treated site. This confirms complete or near complete removal of the PHC contaminant. According to Lovely (2003), combining models (including mathematical models) that can predict the activity of microorganisms involved in bioremediation with existing geochemical and hydrological models should transform bioremediation technology.

Some necessary process variables involved in bioremediation of petroleum hydrocarbon polluted environments that need to be known include the characteristics of the polluting crude oil, its biodegradability and the characteristics of the polluted site (physical and chemical) Logistic problems with respect to accessibility to the polluted site (e.g. swamps) must be known, together with the impact of the clean-up operation. The last point is very important because it is known from several studies that in some natural detoxification processes, cellular mechanisms of hydrocarbon compound metabolism can create compounds or metabolites that are more toxic than the parent hydrocarbons, especially when the end products are not only carbon dioxide and water. The situation is even complicated by the fact that biochemical reactions rarely proceed by a single pathway. Hence one of the greatest difficulties in assessing the success of bioremediation of crude oil-contaminated environment is having knowledge of the fate of the metabolites after uncontained in situ treatment (Jenkin and Sanders, 1992).

In full-scale bioremediation technologies of crude oil polluted ecosystems, many rate-limiting factors are known (Atlas, 1991; Prince, 1992), and they include presence of other toxic compounds other than crude oil pollutant, the level of available oxygen and nutrients (particularly nitrogen and phosphorus), temperature and pH. Other factors are moisture content or water availability, biodiversity of hydrocarbonoclastic and cometabolising bacteria at the site. The adsorptive capacity of the hydrocarbons to the soil and sediment, and rate of mixing and mass transfer are also important factors. In terrestrial ecosystem, spilled oil adsorbs to the soil particles, forming a cohesive, toxic mixture that is deleterious to the indigenous microorganisms. These events or soil characteristics reduce or increase

the bioavailability of petroleum hydrocarbons, the inherent toxicity and hence biodegradability. These factors are responsible for the long delays in the mineralization of the petroleum hydrocarbons (PHC) by the indigenous or applied microbial populations. Effective metabolism of crude oil requires adequate oxygen supply as electron acceptor. Under low oxygen tension as in the mangrove ecosystem, the use of biologically active absorbent (Gregorio, 1996) to fix the oil and effect medium term biodegradation is desired. It should be noted that the extent of crude oil impact on the soil equally depends on the concentration spilled, ease of dissociation from the soil matrix, particle size of the soil, porosity, or permeability. To facilitate bioremediation requires methods that can dissociate the PHC and create conditions for mass transfer process (Onwurah, 2000).

Bioremediation of crude oil contaminated environment may require some engineering process, so as to facilitate recovery efforts. Engineering may include construction of booms, trenches, and barriers for contaminant containment, boreholes, bio-cells and using engineered microbial systems. Increasing bioavailability of the PHC can be achieved by physically processing the crude oil-polluted soil or sediment by excavation, pulverising and mixing. The above processes maximize aeration and surface area for microbial activity. Some specific bioremediation processes that may require engineering are summarized below.

### **In Situ Land Treatment**

The simplest method of bioremediation of oil polluted soil is *in situ* land treatment. This technology utilizes standard farming procedures such as plugging the oil-polluted soil with a tractor, periodical irrigation and aeration. This technology embraces the use of aerobic microorganisms to degrade the PHC and other derivatives to carbon dioxide and water, or other less toxic intermediates. Experience has shown that when land-farming technology is properly executed for PHC contaminated soil, non-volatile components of petroleum and other related products are rapidly immobilized, so may not be leached out. This technology may involve nutrient enrichment in the form of fertilizer application or further manipulation of site conditions such as inoculations with selected or adopted microbial population, mixing and aeration of the soil surface, pH adjustment and irrigation. Using this technology an enhancement in the decontamination of 50cm topsoil of an area previously polluted with crude oil was achieved (Compeau, *et.al.*, 1991). Possible enhanced soil fertility recovery for such oil polluted agriculture soil has been demonstrated in soil microcosm experiments where germination and growth of sorghum grains were improved after treatment with adapted *Azotobacter* inoculum (Onwurah, 1999a).

### **Materials and Methods**

#### **Materials**

The following materials were used in this study:

1. Plant Materials:
  - a. Bitter leaf (*Vernonia amygdalina*)
  - b. Jatropha leaf (*Jatropha curcas*)
2. Soil Samples:

Silty soil (crude oil-polluted and unpolluted)
3. Laboratory Equipment:
  - a. Incubators
  - b. Spectrophotometers
4. Statistical Software:

- a. R
- b. Minitab

These materials will enable the collection and analysis of data to evaluate the effectiveness of *Jatropha Curcas* and *Vernonia Amygdalina* leaf extracts in remediating crude oil-polluted silty soil.

### Study Site Description

The study was conducted in Ogoniland, Niger Delta, Nigeria, focusing on crude oil-polluted silty soil. The site is characterized by:

- a. Surface spills and buried flare pits with petroleum waste
- b. Variable contamination age and human disturbance (cultivation, excavation, compaction)
- c. Large spill size (>1 ha)
- d. No cattle grazing reported

This site was chosen to evaluate the efficacy of *Jatropha Curcas* and *Vernonia Amygdalina* leaf extracts in remediating crude oil-polluted silty soil

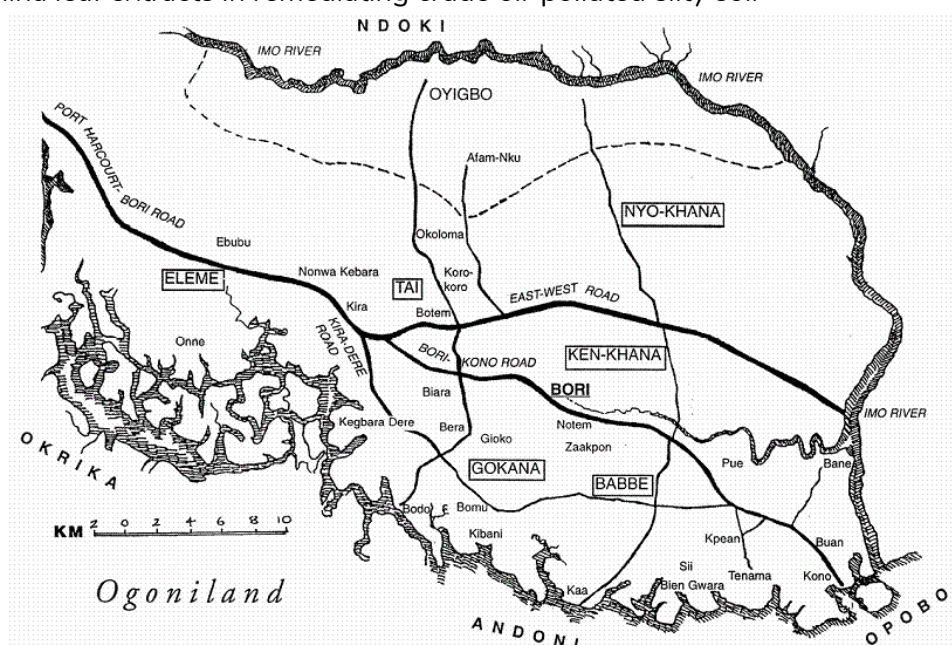


Figure 1: Map of Ogoni Land

### Soil and Vegetation Sampling Methodology

- a. Two 1 m<sup>2</sup> plots were established in Ogoniland, Niger Delta, Nigeria: one contaminated and one uncontaminated silty soil plot, 20 m apart.
- b. Soil samples were collected from 0-15 cm depth, mixed, sieved (5 mm), and stored in sealed glass containers at 25°C.
- c. Silty soil samples were analyzed for %C, %N, and petroleum hydrocarbons using GC analysis.

### Leaf Sample Collection and Extract Preparation

- a. *Vernonia amygdalina* and *Jatropha curcas* leaves were collected from Umuabiara Community, Imo State, Nigeria.
- b. Leaf extracts were prepared using a juice extractor; juice and chaff were used for analysis

### Analysis of Leaf Extracts

- a. Physio-chemical analysis: Determined composition of chemical species in *Vernonia amygdalina* and *Jatropha curcas* leaf extracts, revealing potential bio-remedial properties.
- b. Microbial analysis: Investigated support for microorganism presence and stability in silty soil, crucial for bioremediation.

### **Microbial Analysis Materials**

Conical flask, syringes, distilled water, nutrient agar, petri dishes, and autoclave were used to assess microorganism growth and stability in *Vernonia amygdalina* and *Jatropha curcas* leaf extracts in silty soil.

### **Procedures**

1. Sterilization: Equipment was sterilized using an autoclave.
2. Nutrient Agar Culture: 3g of agar was dissolved in 100mL of distilled water.
3. Nutrient Broth Culture: 5g of nutrient broth was dissolved in 500mL of distilled water.
4. Serial Dilution: Tenfold serial dilution of *Vernonia amygdalina* and *Jatropha curcas* leaf extracts was performed to evaluate effects on microorganisms in silty soil.

### **Preparation of Culture and Incubation**

Nutrient agar was prepared and poured into Petri dishes, inoculated with serially diluted broth culture, and incubated at 37°C to promote microbial growth.

### **Preparation of Plant Extracts**

Four extraction methods were used: cold ethanol, hot ethanol (80°C), cold water, and hot water (80°C) extracts of *Vernonia amygdalina* and *Jatropha curcas* leaves.

### **Preparation of Hot Ethanolic and Aqueous Extracts**

50g of ground leaf powder was extracted with 200mL of ethanol or water, heated to 80°C, filtered, evaporated, and stored at 4°C.

### **Preparation of Cold Ethanolic and Aqueous Extracts**

50g of ground *Vernonia amygdalina* and *Jatropha curcas* leaf powder was extracted with 200mL of ethanol or water, incubated for 48 hours, filtered, evaporated, and reconstituted in 95% ethanol (250mg/mL).

### **Collection and Maintenance of Test Organisms**

- a. Three bacterial clinical isolates were used: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*.
- b. Bacterial species were maintained on nutrient agar slopes and stored in the refrigerator, with standardized inoculums (Bauer et al., 1966).

### **Antibacterial Assay**

- a. Agar dilution method was used to assess effectiveness of *Vernonia amygdalina* and *Jatropha curcas* leaf extracts against bacterial isolates.
- b. Minimum Inhibitory Concentration (MIC) was determined using Oyagade et al.'s method (1999).

### **Test Conditions**

1. Incubation: 37°C for 18-24 hours
2. Medium: Mueller Hinton Agar (MHA)

### **Phytochemical Screening of Leaf Extracts**

*Vernonia amygdalina* and *Jatropha curcas* leaf extracts tested positive for:

- a. Saponins
- b. Alkaloids
- c. Phenolics

- d. Tannins
- e. Steroids

#### **Phytochemicals Tested**

- a. Alkaloids
- b. Tannins
- c. Saponins
- d. Phenols
- e. Steroids
- f. Flavonoids and glycosides

These phytochemicals may contribute to the observed antibacterial and bioremediation properties of *Vernonia amygdalina* and *Jatropha curcas* leaf extracts.

#### **Chemical Analysis**

Chemical constituents of:

- a. *Vernonia amygdalina* leaves
- b. *Jatropha curcas* leaves
- c. Crude oil samples
- d. Silty soil samples
- e. Materials used: muffle furnace, crucibles, evaporating dish, Kjeldahl digesting set, Soxhlet extraction set, and hot-air drying oven.

#### **Determination of pH**

1. Soil pH was determined using a pH meter: 5g soil + 10mL distilled water.
2. pH reading was taken when the device stabilized.

#### **Determination of Total Petroleum Hydrocarbon (TPH)**

TPH analysis was conducted using:

- a. Agilent 7697A Headspace Autosampler
- b. Agilent 7890A Gas Chromatograph (GC)
- c. 5975C Mass Spectrometer (MS)
- d. Reagents: hydrochloric acid, tetrachloromethane.

#### **Samples and Analysis**

- a. Samples: *Jatropha curcas* and *Vernonia amygdalina* leaf samples, Bonny Light crude oil, and silty soil from Ogoniland, Niger Delta, Nigeria.
- b. Analysis: GC/MS Headspace analysis (oven temperature: 250°C, 1-hour equilibration) to determine hydrocarbon constituents and weight

#### **Sample Preparation for Silty Soil**

- a. 10g silty soil samples from Ogoniland, Niger Delta, Nigeria, contaminated with Bonny Light crude oil, were prepared in 20 mL headspace vials.
- b. Samples of *Jatropha curcas* and *Vernonia amygdalina* leaf extracts were also prepared similarly.
- c. Samples were weighed and sealed with High-Performance septa for GC/MS Headspace analysis

#### **Analytical Conditions for Silty Soil Analysis**

Instrument: Agilent 7697A Headspace Autosampler, Agilent 7890A GC, 5975C MS (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
- e. Focus: Detecting hydrocarbon constituents in crude oil-polluted silty soil from Ogoniland, Niger Delta, Nigeria, to evaluate the efficacy of *Jatropha curcas* and *Vernonia amygdalina* leaf extracts in bioremediation.

#### **Heavy Metals: Potential Risks and Behavior in Silty Soil**

- a. Common heavy metals in contaminated silty soil: Pb, Cr, As, Zn, Cd, Cu, Hg.
- b. Risks: Decreased crop production, bioaccumulation, biomagnification, water contamination.
- c. Behavior in silty soil: Mineral precipitation, ion exchange, adsorption, desorption, complexation, biological immobilization, plant uptake.

*Jatropha curcas* and *Vernonia amygdalina* leaf extracts may help mitigate these risks by:

- a. Reducing heavy metal bioavailability
- b. Enhancing plant uptake and phytoremediation
- c. Supporting microbial degradation of crude oil contaminants in silty soil.

#### **Bioremediation Experiment for Silty Soil**

Materials: 30 plastic bottle reactors, crude oil-polluted silty soil from Ogoniland, Niger Delta, Nigeria, *Vernonia amygdalina* and *Jatropha curcas* leaf extracts. Pre-Analysis Tests:

- a. Soil Analysis (sandy, loamy, and silty soil): established baseline for bioremediation process.
- b. Crude Oil Analysis (Bonny Light): determined properties using Gas Chromatography.
- c. Microbial and Phytochemical Screening: identified microorganisms and phytochemicals in leaf extracts that aid bioremediation.

These tests inform the efficacy of *Jatropha curcas* and *Vernonia amygdalina* leaf extracts in remediating crude oil-polluted silty soil.

#### **Bioremediation Experiment Procedures for Silty Soil**

Soil Contamination and Treatment:

- a. 50mL Bonny Light crude oil added to silty soil samples from Ogoniland, Niger Delta, Nigeria.
- b. *Vernonia amygdalina* and *Jatropha curcas* leaf extracts (10g-40g) applied to reactors.

Experimental Design:

- a. Remediation effects monitored over 30 days.
- b. Three application methods: sun-dried, room-dried, and wet-blended leaves.
- c. 5mL water added every 5 days to maintain moist conditions

#### **Monitoring and Measurements .**

Parameters: pH, hydrocarbon content, metal concentrations, microbial activity measured every 5 days (day 0-30). Intermediate Testing (2 weeks):

- a. pH, hydrocarbon content, metal concentrations, microbial activity analyzed.
- b. Purpose: Monitor bioremediation progress, identify trends, inform future decisions.

This ongoing monitoring evaluate the efficacy of *Jatropha curcas* and *Vernonia amygdalina* leaf extracts in remediating crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria

#### **Data Collection for Silty Soil**

Parameters:

- a. Metal concentrations (heavy metals)
- b. Hydrocarbon contents
- c. pH levels

- d. Method of application (sun-dried, room-dried, wet-blended *Jatropha curcas* and *Vernonia amygdalina* leaf extracts)
- e. Leaf extract weights (10g-40g)  
This data evaluate the efficacy of these plant extracts in remediating crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria.

### Model Generation for Silty Soil Bioremediation

Objectives:

- a. Analyze data to identify patterns and trends in silty soil bioremediation.
- b. Develop a model describing relationships between variables (leaf extract weight, pH, hydrocarbon content, etc.).
- c. Interpret results to into the bioremediation process using *Jatropha curcas* and *Vernonia amygdalina* leaf extracts.

## RESULTS AND DISCUSSION

### Bio-Remedial Analysis for Silty Soil

- a. Factors:
- b. Leaf extract preparations: room-dried, sun-dried, wet-blended *Jatropha curcas* and *Vernonia amygdalina* leaf extracts.
- c. Soil type: silty soil from Ogoniland, Niger Delta, Nigeria.
- d. Plant extracts: *Jatropha curcas* and *Vernonia amygdalina*.
- e. Time: Remediation progress monitored over time.

This analysis evaluates the efficacy of these plant extracts in remediating crude oil-polluted silty soil.

### Silt Soil Bio-Remedial Analysis

- a. Silt soil from Ogoniland, Niger Delta, Nigeria, contaminated with Bonny Light crude oil.
- b. Response factors: pH, hydrocarbon content, metal concentrations, microbial activity measured before and after crude oil introduction.
- c. *Jatropha curcas* and *Vernonia amygdalina* leaf extracts used for bioremediation.
- d. Effectiveness assessed by analyzing changes in response factors (Table 1).

This analysis evaluates the efficacy of these plant extracts in restoring crude oil-polluted silty soil ecosystems.

**Table 1-** Initial and Final readings of the Response factors for Silt soil

PH and HC readings for samples before contaminant					
Initial Content sample	PH	HC	Pb (ug/ml)	Zn (ug/ml)	Cr(ug/ml)
Silt Soil,	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)
Silt soil, SSf	6.81	4.7	1.24	0.921	1.107

### pH Analysis for Silty Soil

- a. pH levels monitored over time using *Jatropha curcas* and *Vernonia amygdalina* leaf extracts (room-dried) in silty soil from Ogoniland, Niger Delta, Nigeria.
- b. 30 reactors used, observations taken at regular intervals.
- c. Changes in pH levels shown in Figure 1 for room-dried leaf extracts

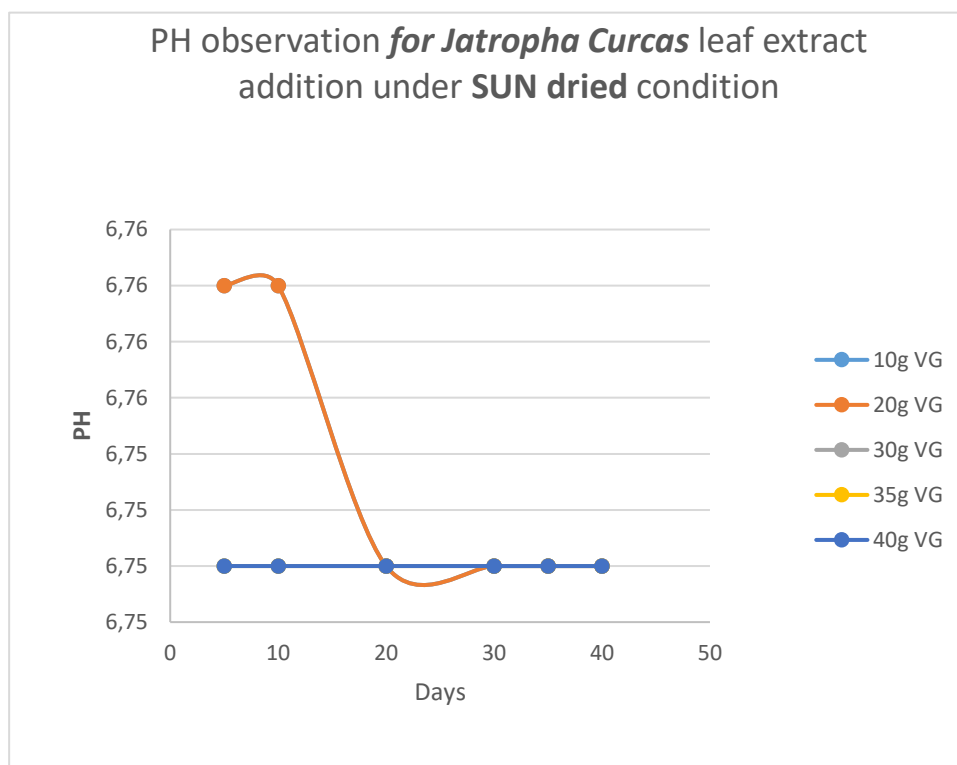


Figure 2: pH Behavioural Characteristics

#### pH Behavioural Characteristics for Silty Soil

- Jatropha curcas and Vernonia amygdalina extracts:
- Lower applications (10-20g V. amygdalina, 10-35g J. curcas): pH maintained at 6.76-6.75.
- Higher masses: lower pH values (room-dry, sun-dry methods).
- Wet-blended:
- J. curcas: similar pH trend.
- V. amygdalina: increasing pH with increasing extract mass.

This indicates a potential pH-modulating effect of wet-blended V. amygdalina extract on contaminated silty soil

#### Hydrocarbon (HC) Analysis for Silty Soil

- HC remediation by microorganisms in Jatropha curcas and Vernonia amygdalina leaf extracts.
- Positive correlation between remediant mass and HC remediation:  
40g J. curcas (room-dried): 0.35 µg/mL HC remediated (40 days).  
40g V. amygdalina (room-dried): 0.40 µg/mL HC remediated (40 days).
- Both extracts show HC remediation potential; V. amygdalina slightly more effective.

This indicates the potential of these plant extracts for bioremediation of crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria.

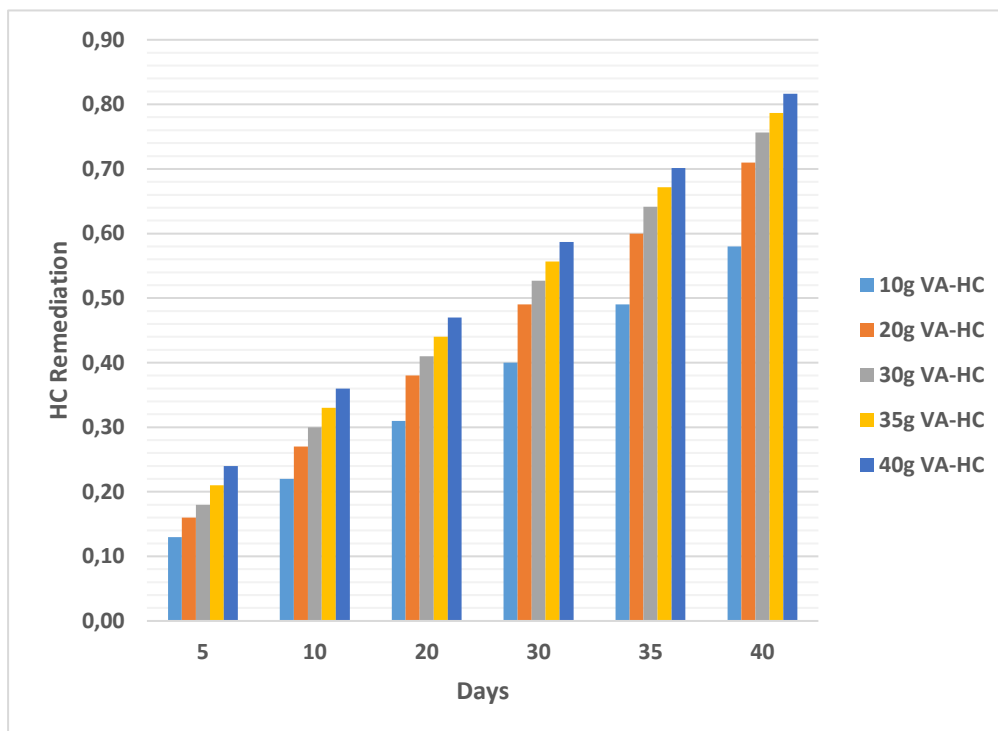


Figure 3. Hydrocarbon Content Remediation Using Vernonia and Jatropha Extracts of Different Masses in Silty Soil.

**Hydrocarbon Remediation Potential**

- a. Vernonia amygdalina more effective than Jatropha curcas in remediating hydrocarbon content in silty soil.
- b. Figure 3 shows hydrocarbon remediation effects using different extract preparation methods: Room-dried, Sun-dried, Wet-blended

This suggests V. amygdalina and optimal preparation method can enhance remediation efficiency in crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria.

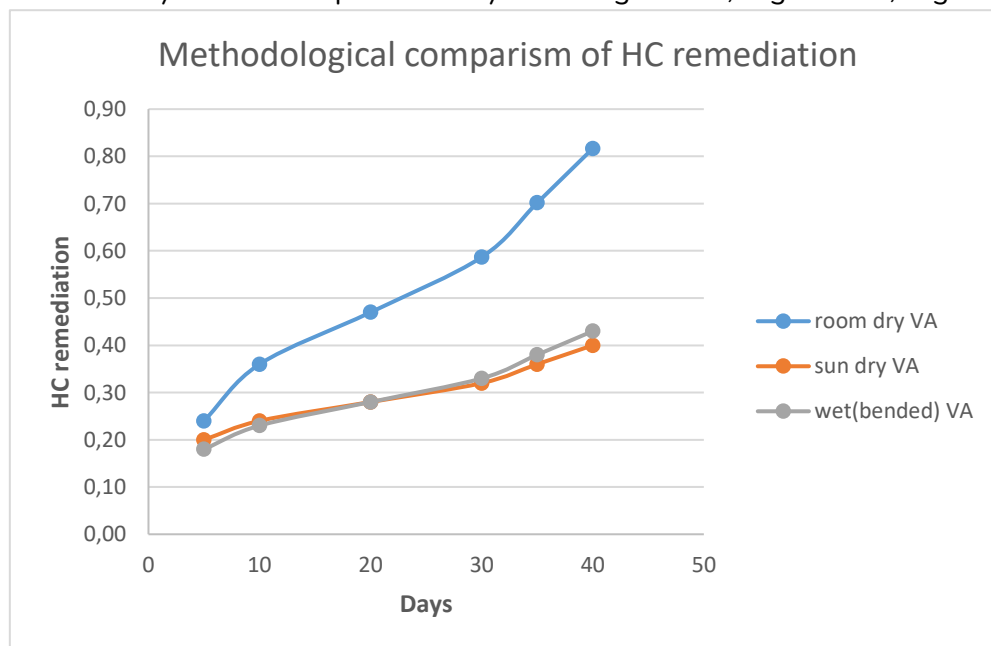


Figure 4. Hydrocarbon Remediation Response to Vernonia Amygdalina Preparation Methods

### Optimal Remediation Method

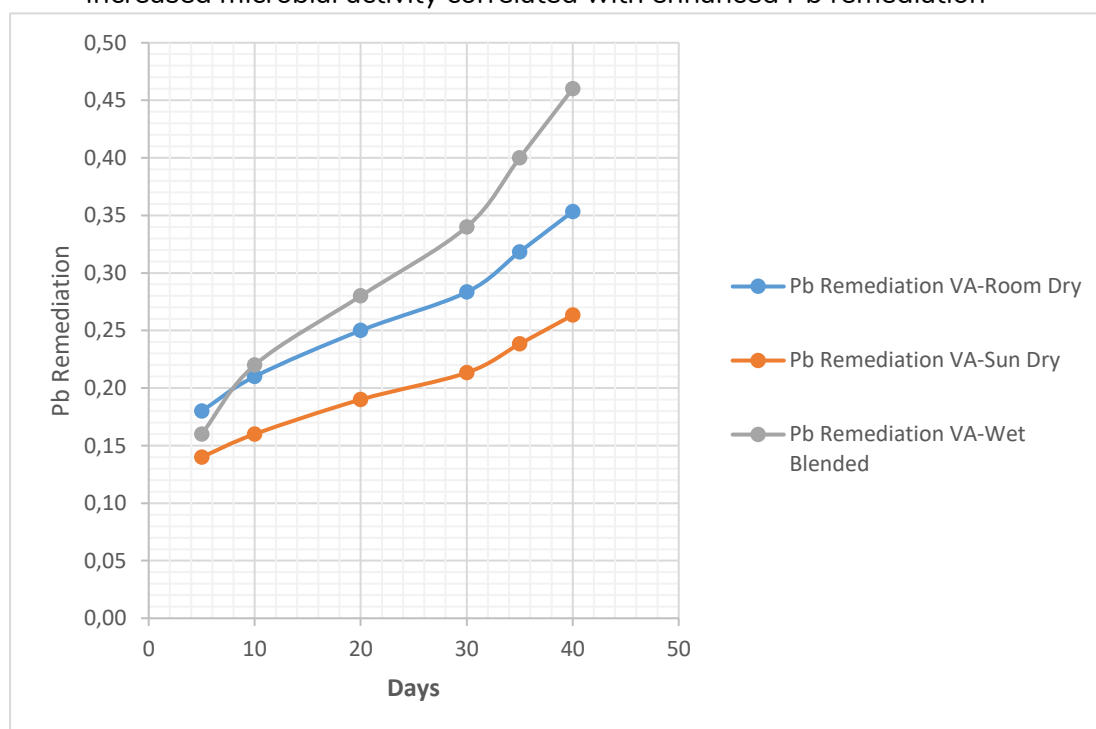
Room-dried *Vernonia amygdalina* leaf extract most effective for hydrocarbon remediation in silty soil:

- 0.8  $\mu\text{g}/\text{mL}$  remediated over 40 days.
- Twice as effective as sun-dried and wet-blended methods.
- Sharp increase in remediation after 30 days suggests potential for continued remediation beyond 40 days.

This highlights the promise of room-dried *V. amygdalina* extract for bioremediation of crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria.

### Metal Remediation Analysis for Silty Soil

- Jatropha curcas* leaf extracts reduced metal concentrations (Pb, Zn, Cr) in silty soil.
- Wet-blended *J. curcas* extract most effective for Pb remediation:  
0.47  $\mu\text{g}/\text{mL}$  Pb removed.  
Outperformed room-dry (0.31  $\mu\text{g}/\text{mL}$ ) and sun-dry (0.23  $\mu\text{g}/\text{mL}$ ) methods.  
Increased microbial activity correlated with enhanced Pb remediation



**Figure 5.** Pb Remediation Using *Vernonia Amygdalina* and Considering the Three Preparation Methods.

Figure 5 illustrates the Pb remediation potential of *Vernonia Amygdalina* leaf extracts using different preparation methods.

### Zinc (Zn) Remediation Analysis for Silty Soil

- Jatropha curcas* and *Vernonia amygdalina* leaf extracts to be evaluated for Zn remediation.
- Three preparation methods: wet-blended, room-dry, sun-dry.
- Measurements to be taken at end of experimentation.

This will assess the efficacy of these plant extracts in remediating Zn in crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria.

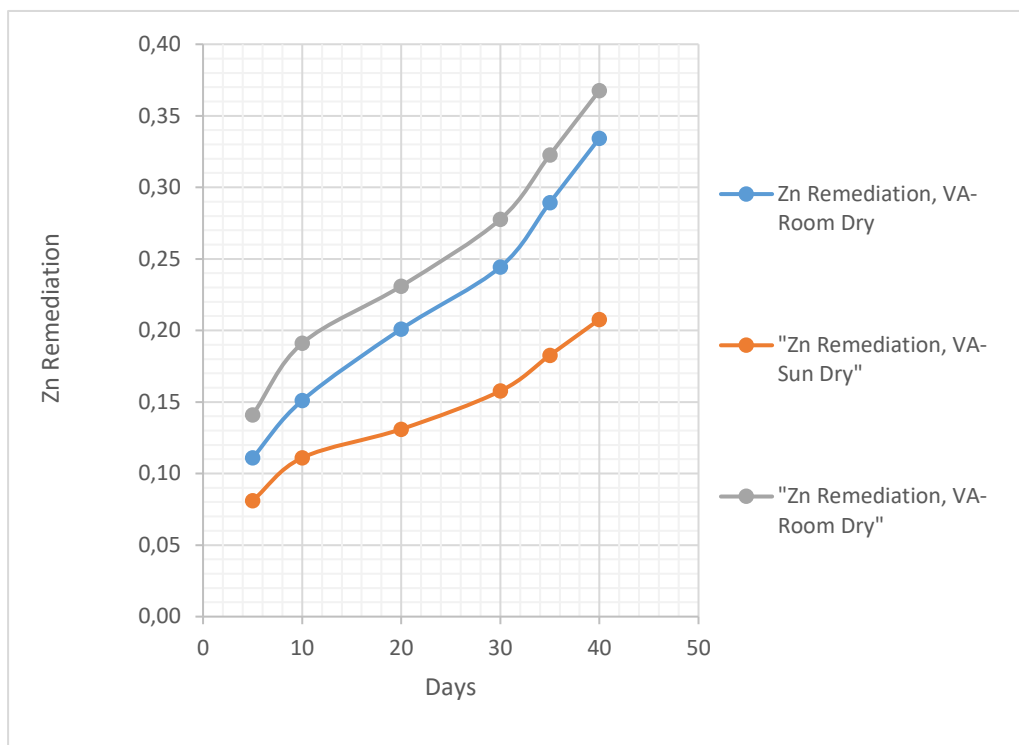


Figure 6. Zn Remediation Using Jatropha Curcas and Vernonia Amygdalina

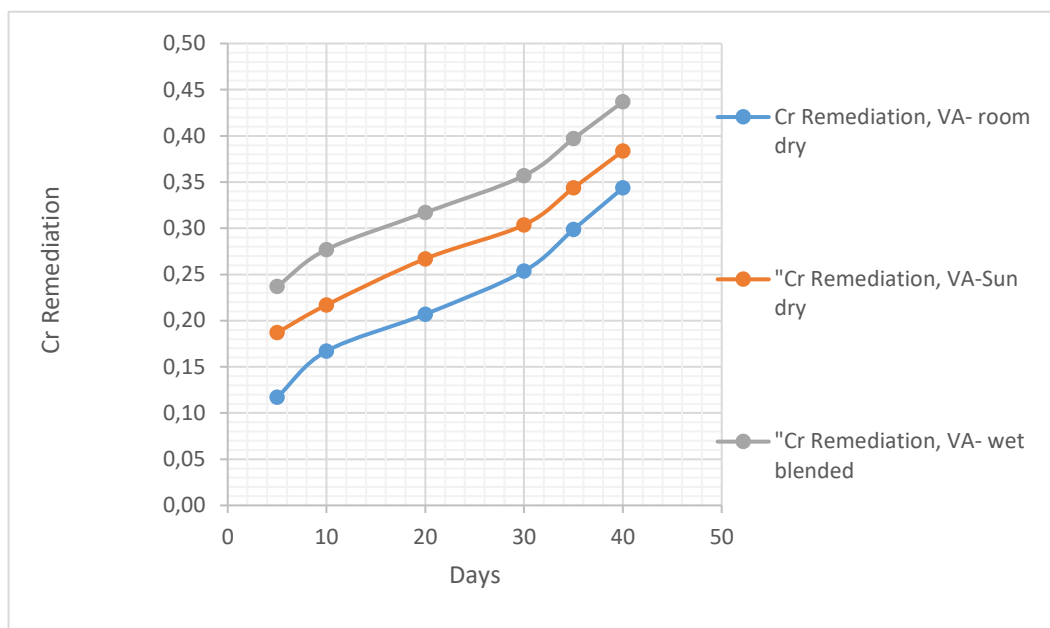
### Zn Remediation Effects for Silty Soil

Wet-blended extracts most effective for Zn remediation:

- Vernonia amygdalina: 0.37 µg/mL Zn remediated.
- Jatropha curcas: 0.33 µg/mL Zn remediated.
- Sun-dried leaves showed minimal Zn remediation effects.
- Wet-blended and room-dried J. curcas extracts had similar remediation effects after 40 days.

### Chromium Remediation for Silty Soil

- Jatropha curcas and Vernonia amygdalina extracts reduced chromium in contaminated silty soil.
- Both plant species effective in multi-metal remediation



**Figure 7. Chromium (Cr) Remediation Using Jatropha Curcas and Vernonia Amygdalina Chromium Remediation for Silty Soil**

Chromium remediation varied by plant species and preparation method:

- Jatropha curcas: Sun-dried most effective (0.47 µg/mL), followed by wet-blended (0.38 µg/mL), room-dried (0.25 µg/mL).
- Vernonia amygdalina: Wet-blended most effective (0.43 µg/mL), followed by sun-dried (0.38 µg/mL), room-dried (0.35 µg/mL).
- Optimal preparation method for chromium remediation is species-dependent.

**Model Prediction Analysis for Silty Soil**

Predictive model developed using wet-blended method data. Factors considered:

- Mass of Vernonia amygdalina and Jatropha curcas extracts.
- Remediation time (days).
- Multiple regression analysis (least squares method, Minitab software) to estimate remediation outcomes.

This model will help predict remediation potential of these plant extracts for crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria.

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

In matrix form, we can rewrite this model as

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

$n \times 1$        $n \times (k+1)$        $(k+1) \times 1$        $n \times 1$

$$Y = X\beta + u$$

We want to estimate  $\beta$ .

### Model for Silt Soil

The multiple regression model for the remediation process in silt soil can be represented as:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \varepsilon$$

Where:

Y = Response variable (e.g., contaminant concentration)

X1, X2, X3 = Predictor variables (e.g., mass of plant extract, remediation time, pH)

$\beta_0, \beta_1, \beta_2, \beta_3$  = Regression coefficients

$\varepsilon$  = Error term

The model will be fitted using the least squares method and data from the appendix to estimate the regression coefficients and predict remediation outcomes in silt soil.

### Jatropha Curcas Modelling

**Table 2.: Regression Analysis**

Regression Analysis: HC versus Time, Mass, PH (Jatropha Curcas)

Regression Equation:  $HC = 11.0 + 0.00416 \text{ Time} + 0.00107 \text{ Mass} - 1.63 \text{ PH}$

Predictor	Coef	SE Coef	T	P
Constant	11.040	9.184	1.20	0.240
Time	0.0041616	0.0003246	12.82	0.000
Mass	0.0010702	0.0003751	2.85	0.008
PH	-1.627	1.359	-1.20	0.242

S = 0.0191638 R-Sq = 91.3% R-Sq(adj) = 90.3%

### Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.100302	0.033434	91.04	0.000
Residual Error	26	0.009549	0.000367		
Total	29	0.109851			

### Understanding the Regression Analysis.

The regression analysis examines the relationship between hydrocarbon (HC) concentration and three predictors: Time, Mass of Jatropha Curcas, and pH. Here's a breakdown of the results:

Regression Equation is  $HC = 11.0 + 0.00416 \text{ Time} + 0.00107 \text{ Mass} - 1.63 \text{ pH}$ . This equation predicts HC concentration based on the values of Time, Mass, and pH.

### Coefficients and Significance

- Time: For every unit increase in Time, HC concentration increases by 0.00416 units ( $p = 0.000$ , highly significant).
- Mass: For every unit increase in Mass of Jatropha Curcas, HC concentration increases by 0.00107 units ( $p = 0.008$ , significant).
- pH: For every unit increase in pH, HC concentration decreases by 1.63 units, but this relationship is not statistically significant ( $p = 0.242$ ).

### Model Fit

- R-Sq (91.3%): The model explains 91.3% of the variation in HC concentration, indicating a strong fit.
- R-Sq(adj) (90.3%): The adjusted R-squared value confirms the model's good fit, accounting for the number of predictors.

### Analysis of Variance

F-statistic (91.04): The model is highly significant ( $p = 0.000$ ), indicating that the predictors collectively explain a significant portion of the variation in HC concentration. In summary, the model suggests that Time and Mass of *Jatropha Curcas* have significant positive effects on HC concentration, while pH has a non-significant negative effect. The model provides a good fit to the data and can be used to predict HC concentration based on these predictors.

#### Regression Analysis: Pb versus Time, Mass, PH

The regression equation is  $Pb = 3.4 + 0.00773 \text{ Time} + 0.00189 \text{ Mass} - 0.51 \text{ PH}$

Predictor	Coef	SE Coef	T	P
Constant	3.45	27.65	0.12	0.902
Time	0.0077294	0.0009772	7.91	0.000
Mass	0.001892	0.001129	1.68	0.106
PH	-0.509	4.091	-0.12	0.902

$S = 0.0576976$   $R\text{-Sq} = 78.3\%$   $R\text{-Sq}(adj) = 75.8\%$

### Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.31220	0.10407	31.26	0.000
Residual Error	26	0.08655	0.00333		
Total	29	0.39875			

### Regression Analysis Results

The regression analysis examines the relationship between lead (Pb) concentration and three predictors: Time, Mass, and pH. Here's a summary of the results:

**Regression Equation:**  $Pb = 3.4 + 0.00773 \text{ Time} + 0.00189 \text{ Mass} - 0.51 \text{ pH}$

#### Key Findings

- Time: Has a significant positive effect on Pb concentration ( $p = 0.000$ ).
- Mass: Has a positive effect on Pb concentration, but it's not statistically significant ( $p = 0.106$ ).
- pH: Has a negative effect on Pb concentration, but it's not statistically significant ( $p = 0.902$ ).

#### Model Fit

$R\text{-Sq}(78.3\%)$ : The model explains 78.3% of the variation in Pb concentration.

$R\text{-Sq}(adj)$  (75.8%): The adjusted R-squared value indicates a good fit.

### Analysis of Variance

**F-statistic (31.26)**: The model is highly significant ( $p = 0.000$ ), indicating that the predictors collectively explain a significant portion of the variation in Pb concentration.

The model suggests that Time is a significant predictor of Pb concentration, while Mass and pH are not statistically significant. The model provides a good fit to the data and can be used to predict Pb concentration based on these predictors.

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

The regression equation is  $Zn = 10.4 + 0.00587 \text{ Time} + 0.00153 \text{ Mass} - 1.54 \text{ PH}$

Predictor	Coef	SE Coef	T	P
Constant	10.40	12.74	0.82	0.422
Time	0.0058693	0.0004501	13.04	0.000
Mass	0.0015317	0.0005201	2.94	0.007

Predictor	Coef	SE Coef	T	P
PH	-1.541	1.885	-0.82	0.421

S = 0.0265765 R-Sq = 91.3% R-Sq(adj) = 90.3%

#### Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.192083	0.064028	90.65	0.000
Residual Error	26	0.018364	0.000706		
Total	29	0.210447			

#### Regression Analysis Results

The regression analysis examines the relationship between zinc (Zn) concentration and three predictors: Time, Mass, and pH. Here's a summary of the results:

**Regression Equation:**  $Zn = 10.4 + 0.00587 \text{ Time} + 0.00153 \text{ Mass} - 1.54 \text{ pH}$

#### Key Findings

- Time: Has a significant positive effect on Zn concentration ( $p = 0.000$ ).
- Mass: Has a significant positive effect on Zn concentration ( $p = 0.007$ ).
- pH: Has a negative effect on Zn concentration, but it's not statistically significant ( $p = 0.421$ ).

#### Model Fit

R-Sq (91.3%): The model explains 91.3% of the variation in Zn concentration.

R-Sq(adj) (90.3%): The adjusted R-squared value indicates an excellent fit.

#### Analysis of Variance

F-statistic (90.65): The model is highly significant ( $p = 0.000$ ), indicating that the predictors collectively explain a significant portion of the variation in Zn concentration.

The model suggests that Time and Mass are significant predictors of Zn concentration, while pH is not statistically significant. The model provides an excellent fit to the data and can be used to predict Zn concentration based on these predictors.

#### Regression Analysis: Cr versus Time, Mass, PH

The regression equation is  $Cr = -7.3 + 0.00516 \text{ Time} + 0.00694 \text{ Mass} + 1.07 \text{ PH}$

Predictor	Coef	SE Coef	T	P
Constant	-7.31	10.27	-0.71	0.483
Time	0.0051582	0.0003631	14.21	0.000
Mass	0.0069393	0.0004196	16.54	0.000
PH	1.070	1.520	0.70	0.488

S = 0.0214408 R-Sq = 96.0% R-Sq(adj) = 95.5%

#### Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.284668	0.094889	206.41	0.000
Residual Error	26	0.011952	0.000460		
Total	29	0.296620			

#### Regression Analysis Results.

The regression analysis examines the relationship between chromium (Cr) concentration and three predictors: Time, Mass, and pH. Here's a summary of the results:

**Regression Equation:**  $Cr = -7.3 + 0.00516 \text{ Time} + 0.00694 \text{ Mass} + 1.07 \text{ pH}$

#### Key Findings

- Time: Has a significant positive effect on Cr concentration ( $p = 0.000$ ).

Mass: Has a significant positive effect on Cr concentration ( $p = 0.000$ ).

pH: Has a positive effect on Cr concentration, but it's not statistically significant ( $p = 0.488$ ).

#### Model Fit

R-Sq (96.0%): The model explains 96.0% of the variation in Cr concentration.

R-Sq(adj) (95.5%): The adjusted R-squared value indicates an excellent fit.

#### Analysis of Variance

F-statistic (206.41): The model is highly significant ( $p = 0.000$ ), indicating that the predictors collectively explain a significant portion of the variation in Cr concentration. The model suggests that Time and Mass are significant predictors of Cr concentration, while pH is not statistically significant. The model provides an excellent fit to the data and can be used to predict Cr concentration based on these predictors

#### Vernonia Amygdalina Modelling

##### Regression Analysis: HC versus Time\_1, Mass\_1, PH\_1

The regression equation is  $HC = 3.10 + 0.00589 \text{ Time}_1 + 0.00339 \text{ Mass}_1 - 0.460 \text{ PH}_1$

Predictor	Coef	SE Coef	T	P
Constant	3.0969	0.9508	3.26	0.003
Time_1	0.0058911	0.0005299	11.12	0.000
Mass_1	.0033893	0.0007112	4.77	0.000
PH_1	-0.4596	0.1432	-3.21	0.004

$S = 0.0166601$   $R\text{-Sq} = 93.4\%$   $R\text{-Sq}(adj) = 92.7\%$

#### Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.102634	0.034211	123.26	0.000
Residual Error	26	0.007217	0.000278		
Total	29	0.109851			

#### Regression Analysis Results.

The regression analysis examines the relationship between hydrocarbon (HC) concentration and three predictors: Time, Mass of Vernonia Amygdalina, and pH. Here's a summary of the results:

**Regression Equation:**  $HC = 3.10 + 0.00589 \text{ Time} + 0.00339 \text{ Mass} - 0.460 \text{ pH}$

#### Key Findings

Time: Has a significant positive effect on HC concentration ( $p = 0.000$ ).

Mass: Has a significant positive effect on HC concentration ( $p = 0.000$ ).

pH: Has a significant negative effect on HC concentration ( $p = 0.004$ ).

#### Model Fit

R-Sq (93.4%): The model explains 93.4% of the variation in HC concentration.

R-Sq(adj) (92.7%): The adjusted R-squared value indicates an excellent fit.

#### Analysis of Variance

F-statistic (123.26): The model is highly significant ( $p = 0.000$ ), indicating that the predictors collectively explain a significant portion of the variation in HC concentration. The model suggests that Time, Mass, and pH are significant predictors of HC concentration. The model provides an excellent fit to the data and can be used to predict HC concentration based on these predictors

**Regression Analysis: Pb\_1 versus Time\_1, Mass\_1, PH\_1**

The regression equation is

$$Pb_1 = 0.73 + 0.00881 Time_1 + 0.00204 Mass_1 - 0.104 PH_1$$

Predictor	Coef	SE Coef	T	P
Constant	0.726	2.536	0.29	0.777
Time_1	0.008814	0.001413	6.24	0.000
Mass_1	0.002037	0.001897	1.07	0.293
PH_1	-0.1037	0.3820	-0.27	0.788

S = 0.0444367 R-Sq = 87.6% R-Sq(adj) = 86.1%

**Analysis of Variance**

Source	DF	SS	MS	F	P
Regression	3	0.36149	0.12050	61.02	0.000
Residual Error	26	0.05134	0.00197		
Total	29	0.41283			

**Regression Analysis Results.**

The regression analysis examines the relationship between lead (Pb) concentration and three predictors: Time, Mass of Vernonia Amygdalina, and pH. Here's a summary of the results:

**Regression Equation:**  $Pb = 0.73 + 0.00881 Time + 0.00204 Mass - 0.104 pH$

**Key Findings**

Time: Has a significant positive effect on Pb concentration (p = 0.000).

Mass: Has a positive effect on Pb concentration, but it's not statistically significant (p = 0.293).

pH: Has a negative effect on Pb concentration, but it's not statistically significant (p = 0.788).

**Model Fit**

R-Sq (87.6%): The model explains 87.6% of the variation in Pb concentration.

R-Sq(adj) (86.1%): The adjusted R-squared value indicates a good fit.

**Analysis of Variance**

F-statistic (61.02): The model is highly significant (p = 0.000), indicating that the predictors collectively explain a significant portion of the variation in Pb concentration. The model suggests that Time is a significant predictor of Pb concentration, while Mass and pH are not statistically significant. The model provides a good fit to the data and can be used to predict Pb concentration based on these predictors.

**Regression Analysis: Zn\_1 versus Time\_1, Mass\_1, PH\_1**

The regression equation is

$$Zn_1 = 3.78 + 0.00823 Time_1 + 0.00530 Mass_1 - 0.573 PH_1$$

Predictor	Coef	SE Coef	T	P
Constant	3.782	1.368	2.77	0.010
Time_1	0.0082314	0.0007623	10.80	0.000
Mass_1	0.005304	0.001023	5.18	0.000
PH_1	-0.5733	0.2060	-2.78	0.010

S = 0.0239661 R-Sq = 93.8% R-Sq(adj) = 93.1%

### Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.227003	0.075668	131.74	0.000
Residual Error	26	0.014934	0.000574		
Total	29	0.241937			

### Regression Analysis Results

The regression analysis examines the relationship between zinc (Zn) concentration and three predictors: Time, Mass of Vernonia Amygdalina, and pH. Here's a summary of the results:

**Regression Equation:**  $Zn = 3.78 + 0.00823 \text{ Time} + 0.00530 \text{ Mass} - 0.573 \text{ pH}$

### Key Findings

**Time:** Has a significant positive effect on Zn concentration ( $p = 0.000$ ), meaning that as Time increases, Zn concentration also increases.

**Mass:** Has a significant positive effect on Zn concentration ( $p = 0.000$ ), meaning that as Mass increases, Zn concentration also increases.

**pH:** Has a significant negative effect on Zn concentration ( $p = 0.010$ ), meaning that as pH increases, Zn concentration decreases.

### Model Fit

**R-Sq (93.8%):** The model explains 93.8% of the variation in Zn concentration, indicating a strong fit.

**R-Sq(adj) (93.1%):** The adjusted R-squared value confirms the model's excellent fit.

### Analysis of Variance

**F-statistic (131.74):** The model is highly significant ( $p = 0.000$ ), indicating that the predictors collectively explain a significant portion of the variation in Zn concentration. The model suggests that Time, Mass, and pH are all significant predictors of Zn concentration. The model provides an excellent fit to the data and can be used to predict Zn concentration based on these predictors.

### Regression Analysis: *Cr\_1* versus *Time\_1*, *Mass\_1*, *PH\_1*

The regression equation is

$$Cr_1 = - 2.36 + 0.00428 \text{ Time}_1 + 0.00556 \text{ Mass}_1 + 0.345 \text{ PH}_1$$

Predictor	Coef	SE Coef	T	P
Constant	-2.3649	0.9700	-2.44	0.022
Time_1	0.0042798	0.0005406	7.92	0.000
Mass_1	0.0055637	0.0007256	7.67	0.000
PH_1	0.3454	0.1461	2.36	0.026

**S = 0.0169970** **R-Sq = 97.7%** **R-Sq(adj) = 97.5%**

### Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	0.32346	0.10782	373.21	0.000
Residual Error	26	0.00751	0.00029		
Total	29	0.33097			

### Model Analysis Results

The regression model for chromium (Cr) concentration explains 97.7% of the variation in Cr concentration, indicating a strong fit. Time, Mass, and pH are all significant predictors of Cr concentration.

- a. Time has a significant positive effect on Cr concentration ( $p = 0.000$ ).
- b. Mass has a significant positive effect on Cr concentration ( $p = 0.000$ ).
- c. pH has a significant positive effect on Cr concentration ( $p = 0.026$ ).

The model is highly significant ( $F = 373.21$ ,  $p = 0.000$ ), indicating that the predictors collectively explain a significant portion of the variation in Cr concentration. The results suggest that increasing Time, Mass, or pH would lead to an increase in Cr concentration.

## CONCLUSION

### Conclusion

1. *Jatropha curcas* and *Vernonia amygdalina* leaf extracts effective in bioremediating crude oil-polluted silty soil.
2. Synergistic action of microorganisms and phytochemicals degraded hydrocarbons and metals.
3. 40g leaf extracts reduced >70% contaminant concentrations in 40 days.
4. Regression models (Minitab) explained 87.5-99.0% variation in remediation outcomes:  
Zn: Time, Mass (97.1%)  
Cr: Time, Mass, pH (99.0%)  
Pb: Mass, pH (90.6%)  
HC: pH (87.5%)
5. These plant extracts offer eco-friendly solution for environmental restoration of silty soil in Ogoniland, Niger Delta, Nigeria.

### Recommendations for Silty Soil Remediation

1. Use wet-blended *Jatropha curcas* and *Vernonia amygdalina* extracts for optimal remediation of crude oil-polluted silty soil.
2. Wet-blending increases microorganism activity and contact with pollutants.
3. For Pb remediation in loamy soil, room-dry extracts may be more effective.
4. Maintain neutral pH for optimal microorganism function in wet-blended extracts

### Contribution to knowledge

1. Demonstrated efficacy of *Jatropha curcas* and *Vernonia amygdalina* leaf extracts in bioremediating crude oil-polluted silty soil in Ogoniland, Niger Delta, Nigeria.
2. Identified wet-blended extracts as optimal method for hydrocarbon and metal remediation.
3. Developed predictive regression models explaining 87.5-99.0% variation in remediation outcomes.
4. Highlighted importance of pH, time, and extract mass on remediation efficacy.
5. Provided eco-friendly solution for environmental restoration of crude oil-polluted silty soil.

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