International Journal of Engineering Research and Development
e- ISSN: 2278-067X, p-ISSN: 2278-800X, www.ijerd.com
Volume 22, Issue 5 (May 2026), PP 166-176

Performance Evaluation of Citrus Fruit Albedo in the Bioremediation of Hydrocarbon-Polluted Soil

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Abstract

Hydrocarbon pollution remains one of the most critical environmental challenges, especially in oil-producing regions such as the Niger Delta, where frequent oil spills and artisanal refining activities have led to severe soil degradation, loss of fertility, and ecological imbalance. Conventional remediation techniques, though effective, are often costly, time-consuming, and environmentally unsustainable. This study investigated the bioremediation potential of citrus fruit albedo, a waste by-product of citrus processing, as an eco-friendly and cost-effective alternative for the treatment of hydrocarbon-contaminated soil. Loamy soil samples collected from Agbonchia in Eleme Local Government Area of Rivers State were artificially contaminated with crude oil and treated with varying quantities (50 g, 100 g, and 150 g) of both sun-dried and room-dried citrus albedo under controlled laboratory conditions for a period of five weeks. Physicochemical parameters including pH, temperature, total dissolved solids (TDS), conductivity, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total hydrocarbon content (THC), and sulphate were monitored at weekly intervals, alongside microbial population counts. The results revealed a significant reduction in total petroleum hydrocarbons across all amended samples compared to the unamended control, with the highest degradation rate observed in soil treated with 150 g of room-dried albedo. Progressive improvements were also recorded in soil pH, moisture content, and oxygen availability, while microbial growth increased markedly, indicating enhanced biodegradation activity. Statistical analysis confirmed that the differences among treatments were significant ($p < 0.05$), establishing a strong correlation between albedo concentration and hydrocarbon degradation efficiency. The findings demonstrate that citrus fruit albedo not only accelerates the breakdown of petroleum hydrocarbons but also improves soil physicochemical conditions, making it a viable bio stimulant for sustainable soil restoration. This study revealed that the utilization of citrus albedo represents a dual environmental benefit- effective remediation of polluted soils and value-added recycling of agricultural waste- offering a practical solution for ecological rehabilitation in oil-impacted regions of the Niger Delta and beyond.

Keywords: *Bioremediation, Hydrocarbon pollution, Citrus fruit albedo, Total petroleum hydrocarbons (TPH), Microbial degredation, Biostimulation, Soil physiochemical properties, Agro waste utilization.*

Date of Submission: 01-05-2026 Date of acceptance: 09-05-2026

I. INTRODUCTION

Hydrocarbon pollution, largely resulting from the exploration, transportation, and utilization of petroleum and its derivatives, remains a significant environmental concern globally. The petroleum hydrocarbons are the examples of enduring organic pollutants that negatively impact the soil ecosystems, reduce agricultural productivity, and threaten soil and water biodiversity (Zhou et al., 2020). In oil rich countries like the Niger Delta of Nigeria, frequent spills, informal refineries and the vandalism of the oil pipes have rendered large areas of land to be heavily contaminated. These contaminated soils are not suitable to grow agricultural products and they also present serious risks to human beings by contaminating ground water and bioaccumulating along the food chain (Chikere et al., 2017; Ehirim and Nwankwoala, 2021). High water insolubility, high molecular weight, microbial insolubility are the complex properties of petroleum hydrocarbons that make them persistent in the environment (Adams et al., 2015). This leaves a strong call to consider coming up with new innovative, sustainable, and cost-effective remediation plans which can help ensure that the ecologically integrity of the polluted environments is put back on track.

The oil spills can be of crude oil or refined oil products like fuel and lubricating oils. A wide range of hydrocarbons, nitrogen-oxygen composites, sulfur compounds, and heavy metals make chemicals of crude oils to cause acute and chronic impacts on plant and animal life (Atlas & Hazen, 2011). Thus, these pollutants should be remedied. Moreover, crude oil comprises of both complex and toxic hydrocarbons, making its cleanup and recovery to be more than challenging. Typically, the treatment methods for disposing contaminated sites include thermal, physical, chemical, and biological processes (Varjani& Upasani 2012). Generally, dependent on the type and quantity of pollution and weather conditions, one or a combination of these techniques is used (Dave & Ghaly 2011). Each technique has its own advantages and disadvantages. The mechanical and chemical methods are often considered as primary methods for quick cleanup and prevention of the oil spreading (Dave & Ghaly 2011). However, their applications require costly equipment and reagents and involve complex processes. They may also subsequently cause mechanical damage or toxic effects on the ecosystem. In comparison with some physical and chemical approaches, biological treatment is considered as a more effective and economical method with less impact to the environment. In biological treatment, microorganisms or plants are used to remove pollutants. This offers the advantages of less labor requirement and potential complete mineralization of oil to CO_2 and H_2O. However, biological treatment can take a long time and is often only applicable when time is not a limiting factor. Also, the application of this method can be limited by abiotic environmental factors such as oil concentrations, nutrients, pH, temperature, and insufficient oxygen (Chatterjee *et al.* 2008).

Bioremediation broadly refers to any process wherein a biological system (typically bacteria, microalgae, fungi in mycoremediation, and plants in phytoremediation), living or dead, is employed for removing environmental pollutants from air, water, soil, fuel gasses, industrial effluents etc., in natural or artificial settings (Sales da Silva *et el.,* 2020). The natural ability of organisms to adsorb, accumulate, and degrade common and emerging pollutants has attracted the use of biological resources in treatment of contaminated environment (Sharma, *et al.,* 2018). In comparison to conventional physicochemical treatment methods bioremediation may offer advantages as it aims to be sustainable, eco-friendly, cheap, and scalable. This technology is rarely implemented however because it is slow or inefficient. Most bioremediation is inadvertent, involving native organisms. Research on bioremediation is heavily focused on stimulating the process by inoculation of a polluted site with organisms or supplying nutrients to promote their growth. Environmental remediation is an alternative to bioremediation (Patel, *et al.,* 2022). It is a process in which the biological pathways within microorganisms, animal products as well as plants are used to degrade or sequester toxic hydrocarbons, heavy metals, and other volatile organic compounds found within fossil fuels. It involves the use of biological products' ability to extract, degrade, stabilize, and volatilize a large array of both organic and inorganic contaminants located in soil and liquid substrates, and air (Kabra *et al.* 2012). Plants, either alone or in conjunction with microorganisms, have been reported to be used successfully for the bioremediation of contaminants. The advantages of bioremediation methods include minimal on-site operational costs, no secondary pollution, and greater public acceptance (Chao *et al,* 2017).

Due to the ubiquity of crude oil across environments, many organisms have evolved to use the hydrocarbons and organic compounds in petroleum as energy while simultaneously denaturing toxins through molecular transfer mechanisms (Hazen, 2018). Microbial bioremediation uses aerobic and anaerobic properties of various microbes to respire and ferment compounds transforming toxins into innocuous compounds. These resulting compounds exhibit more neutral pH levels, increased solubility in water, and are less reactive molecularly. Baseline populations of oil-degrading microorganisms typically account for less than 1% of microbiomes associated with marine ecosystems (Garbisu, &Alkorta, 2013). Remediation techniques which remove reaction limiting factors through the addition of substrate, can boots microbe population towards 10% of the ecosystems microbiome. Dependent on physical and chemical properties, petroleum-degenerative microorganisms take longer to degrade compounds with high-molecular-weight, such as polycyclic aromatic hydrocarbons (PAH's). These microbes require a wide array of enzymes for the breakdown of petroleum, and very specific nutrient compositions to work at an efficient rate. Microbes work in a step-wise fashion to breakdown and metabolize the components of petroleum.

Citrus fruits such as oranges (*Citrus sinensis*), lemons (*Citrus limon*), and grapefruits (*Citrus paradisi*) are globally consumed in large quantities, generating vast amounts of peel waste during processing. These peels find their way into landfills thus contributing to emission of greenhouse gases and generation of leachates (Rafiq et al., 2016). The albedo, which is the inner white part of the peel is a source of pectin, cellulose, hemicellulose and essential oils (Ayala-Zavala et al., 2011). The structural and chemical constituents present in these soil layers gives an optimal substrate to the growth of microorganisms and their activity, which increases the breakdown of petroleum hydrocarbons in contaminated soils. Citrus albedo is also rich in carbon and has a porous structure, which enhances soil texture, aeration, and the retention of moisture that are important in successful degradation by microbes (Kumar et al., 2017). Moreover, the albedo has a diversity of bioactive compounds that have been reported to have antimicrobial, antioxidant, and enzymatic activating properties, which can influence the structure of microbial communities and can catalyze biotransformation reactions (Gonzalez-Molina et al., 2010). These phytochemicals can ensure the growth of hydrocarbon-degrading microorganisms and inhibit the feature of pathogens that carry out the competition. Regardless of these possible advantages, few studies have been done on the use of citrus albedo in bioremediation. The majority of the literature has been assigned to commercial valorization of citrus wastes to extract pectin, use it as animal feed, or as a bioenergy source (Ciriminna et al., 2017), but comparatively little has been paid to its potential of environmental remediation especially in comparative performance evaluation to other amendments.

The application of bioremediation is one of the most prospective and ecologically upright mechanisms of treating the hydrocarbon-contaminated soils. It entails application of biological agents like bacteria, fungi, plants and plant based materials to degrade or detoxify or convert harmful contaminants into less toxic products (Das & Chandran, 2019). Organic waste amendment is one of the bioremediation methods, which have received significant focus because it can activate the activity of native microbial groups, enhance soil aeration, and increase the bioavailability of hydrocarbons (Onuoha et al., 2016). Organic matter Compost, animal manure and agricultural by-products are sources of nutrients that enhance metabolism of microorganisms and degradation of pollutants. In the recent past, there has been an increasing interest in the use of fruit wastes especially citrus peels as possible biostimulants in polluted environments.

This research, thus, aims at determining the effectiveness of citrus fruit albedo in cleaning up in the bioremediation of the soil affected by the hydrocarbon. The main objective is to identify how citrus albedo can exert positive effects to both microbial activities, speed-up in the process of reducing the total petroleum hydrocarbons (TPH) concentration and improve characteristics of physicochemical properties of polluted soils.

Bento et al. (2005) was one of the pioneering studies in this field; the authors compared three bioremediation practices namely natural attenuation, biostimulation, and bioaugmentation of the diesel-contaminated soils. They found out that biostimulation (with the introduction of nitrogen and phosphorus nutrients) boosted the microbial degradation of hydrocarbons significantly as compared to the natural attenuation and bioaugmentation. The results highlighted the significance of the availability of nutrients to better biodegradation activity in the favour of best microbial activity. They concluded that biostimulation is a cost-effective tool particularly where there were indigenous populations of microbes with hydrocarbon degradation capacity, and advised its extensively to be used in field remediation efforts, in soils with nutrient deficiency.

Okoh, Trejo-Hernandez and Uzochukwu (2011) have examined how microbial consortia are used to eliminate hydrocarbon bioremediation. In their research, it was established that mixed microbial populations, more so those that incorporated Pseudomonas, Bacillus and Acinetobacter, exhibited greater efficiency of degradation as opposed to single strains. The complementary attack on a broader range of hydrocarbon fractions was possible as a result of the synergy between the various microbial species. The authors estimated that consortia-based bioremediation is more robust and efficient, especially with complex or grossly polluted locations, and suggested developing specialised consortia with reference to particular contamination profiles so that specialised consortia may be created or applied in the field with its more efficient work.

Similarly, Ijah, Safiyanu, and Abdulrahaman (2014) examined the remediation of crude-oil-contaminated soil based on the organic (poultry droppings) and inorganic (urea) amendments. Their findings showed that the growth and degradation of microbes of poultry manure was much greater and quicker than the urea in total petroleum hydrocarbons (TPH). This was affirmed by the fact that a wider spectrum of nutrients and organic matter of poultry manure favored a wider and diverse microbial community. This study established that organic amendments can in addition to improving the efficiency of bioremediation, they also play a role towards the long-term soil fertility and the authors suggested the incorporation of locally sourced organic wastes in community-level remediation programmes.

Akinola, Ogundipe, and Abolusora (2021) assessed agro-wastes such as banana peels and plantain skins as biological remedies in the soil more recently. The analysis of their work demonstrated that the materials enhanced the activity of microorganisms and the rate of hydrocarbon breakdown. They determined that agro-waste amendments stimulated the aeration of the clay soils and the availability of microbial nutrients resulting in the over 60 percent TPH degradation after a duration of 28 days. The authors have concluded that agro-waste valorisation can be not only of use to the environment but also to resource-limited areas, and still proposed additional research on the particular types of waste and conditions of decomposition in order to achieve the most positive results of the remediation process.

Citrus waste (especially, albedo of citrus peels) has also been tested on remediation potential. According to Adewoye, Olatunji, and Bello (2023), citrus albedo showed remarkable improvement in hydrocarbon degradation in polluted soils where more than 65% of total mass reduced in 30 days. They explained this by the fact that the albedo contained a great amount of pectin and cellulose which are the sources of necessary nutrients and nutrient-driving microbes. The result of the study was that citrus waste is a good and sustainable biostimulant and that should be used in regions having a high production and processing of citrus.

In a similar type of research, Uzoije and Agunwamba (2023) focused on biostimulatory activities of orange peel on the dynamics of microbial populations in the oil-oil-contaminated soils. Their results revealed that there was an enhanced diversity of microbes, enzyme activity, and accelerated hydrocarbon degradation in the amended soils as compared to the controls. They decided that orange peel is a good organic amendment to use and suggested its application in low-cost remediation structures, especially in urban and peri-urban agricultural lands.

Also, Ndukwe, Ubah, and Onwukwe (2024) examined the synergetic effect of citrus waste when fed together with phytoremediation by use of *Mucuna pruriens. In their field-based experiment, they demonstrated that this biological combination enhanced the level of hydrocarbon degradation and soil restoration. The rhizosphere was provided with citrus refuse which was a microbial stimulant and legume input which was found to fix nitrogen. This research inference was that integrated phytobioremediation is one of the potential ways through which land could be recovered in a sustainable manner, and the researchers suggested the application of such technology in the Niger Delta and other oil-polluted areas in sub-Saharan Africa to fully rehabilitate the ecology of the environment.

Ehirim et al. (2020) conducted research to investigate a mixed-model formulation of TPH prediction in the process of bioremediation of hydrocarbon-contaminated soils. As the bioremediation of polluted soil has a number of input parameters that must be satisfied in ensuring an acceptable goal is obtained, this paper came up with a mix-design model that can be used to forecast the quantity of total petroleum hydrocarbon (TPH) that will be eliminated in a given soil contaminated with crude oil by the bioremediation process. Thus, the percentage of TPH removed was expressed as a function of treatment time, treatment dosage, crude oil volume and soil weight. The leaves and barks of mango (Magnifera indica) were used as amendment agents. The leaves and bark of mango were separately crushed to fine particle sizes and sieved to 2.0mm. Sandy, silt loam and clay soils were used for this study. The TPH content was analyzed every 7 days for a total period of 28 days. The results obtained showed that increase in time and treatment weights also increases the percentage of TPH removed from the soils. However, sample with treatments recorded highest amount of TPH degradation than the control samples, and it was highest with 50g treatment weight. For instance, at the 28th day of analysis for 50g treatment weight, the TPH percentage removed from sandy, silt loam and clay soils with mango leave particles were obtained as 93.84%, 91.87% and 96.93% respectively, while with mango bark, the TPH percentage removed were 94.49%, 92.37% and 97.13% for sandy, silt loam and clay soils respectively. Thus, the results implied that mango bark treatment slightly outperformed mango leaves in reducing TPH content in the soils, while the highest degradation of TPH was recorded in clay soil, followed by sandy soil and least in silt loam soil. Finally, it is shown that the correlation coefficient between the measured and the predicted percentages of TPH removed from sandy, silt loam and clay soils treated with mango leaves and bark ranged from 0.9225 to 0.9613, which indicated that over 90% of the measured TPH was explained by the mix design model. Hence, the mix model can be applied to estimate the optimum treatment weight and time required to remediate a given weight of soil polluted with a known volume of crude oil.

Ukpaka, (2018), carried out an investigation to demonstrate the integration of improved oil palm fiber (Tekena Species) dried in dark environment for the remediation of loamy soil polluted with crude oil. Analysis was conducted to determine the characteristics of the effectiveness of the improved oil palm fibre (Tekena Species) dried in dark environment on the degradation of the crude oil in loamy soil environment using X-ray fluorescence spectrometer (GC).The following elements were identified from the Tekena Species Mg, P, Si, S, K, and Ca were obtained within the energy level of > 0 to < 250J, Ti, Mn, Fe, Co, Ni, Cu and Zn with energy level range of > 250J to < 590J, W, Au, Pb, Rb, Zr, Nb, and Mo with energy level range of > 600J to< 1200J and Ag, Cd, Sn and Sb with energy level range of > 1400J to < 1800J. The micro-organism isolated and identified were six different fungi species with a population of 1.2x105CFU.g-1 for Tekena Species. The bacteria isolated and identified five different species with a population of 9.0x106CFU.g-1 for Tekena Species. It is observed that species are very effective when used for bioremediation of polluted soil environment. The Total Petroleum Hydrocarbon (TPH) in the loamy soil sample was examined for 0 to 84 days to ascertain the degree of degradation upon the influence of improved oil palm fiber (Tekena Species) dried in dark environment and the characteristics to improve the level of restoration of the polluted loamy soil was encouraging. A model was developed to determine the rate of degradation of pollutants with time. The result from the model validates the experiment with improved oil palm fiber (Tekena Species) dried in dark environment and the degree of degradation is rated in percentage as 76.45%.

Ali, *et al.,* (2020) carried out a study on Bioremediation of soils saturated with spilled crude oil. A desert soil sample was saturated with crude oil (17.3%, w/w) and aliquots were diluted to different extents with either pristine desert or garden soils. Heaps of all samples were exposed to outdoor conditions through six months, and were repeatedly irrigated with water and mixed thoroughly. Quantitative determination of the residual oil in the samples revealed that oil-bioremediation in the undiluted heaps was nearly as equally effective as in the diluted ones. One month after starting the experiment. 53 to 63% of oil was removed. During the subsequent five months, 14 to 24% of the oil continued to be consumed. The dynamics of the hydrocarbon clastic bacterial communities in the heaps was monitored. The highest numbers of those organisms coordinated chronologically with the maximum oil-removal. Out of the identified bacterial species, those affiliated with the genera Nocardioides (especially N. deserti), Dietzia (especially D. papillomatosis), Microbacterium, Micrococcus, Arthrobacter, Pseudomonas, Cellulomonas, Gord onia and others were main contributors to the oil consumption. Some species, e.g. D. papillomatosis were minor community constituents at time zero but they prevailed at later phases. Most isolates tolerated up to 20% oil, and D. papillomatosis showed the maximum tolerance compared with all the other studied isolates. It was concluded that even in oil saturated soil, self-cleaning proceeds at a normal rate. When pristine soil receives spilled oil, indigenous microorganisms suitable for dealing with the prevailing oil-concentrations become enriched and involved in oil-biodegradation.

Ahmad, *et al.,* (2020), carried out a study on remediation methods of crude oil contaminated soil. The authors stated that soil contamination due to crude oil leakage has adverse effects on human and vegetation growth so its removal is essential. Many methods have been developed to remove crude oil from the soil i.e., physical, chemical, thermal and biological. Many alterations and development have been introduced in Physio-chemical and thermal methods to enhance their efficiency and reduce their demerits. Still these methods have many drawbacks and less acceptable by the society. On the other hand, bioremediation methods are preferred because they are efficient, cheap and nature friendly. In the recent technology i.e., rhizoremediation, microbes and plants are combined together in synergistic relationship to efficiently remove the crude oil contaminants from the soil. Research has shown that rhizoremediation is more efficient than microbial and phytoremediation techniques separately. In this review, different remediation techniques to remove crude oil from the soil have been discussed, focusing on their current advancement. Chemical, physical and thermal methods used for the cleanup of soil have many demerits, so focus is shifted toward biological methods such as microbial remediation and phytoremediation. Recently microbes and plants are used together as rhizoremediation technique to remove contaminants from the soil because of its significant results.

Ndimele, *et al.,* (2018) worked on Remediation of crude oil spillage. Oil spillage is a crucial environmental catastrophe of universal interest. Occurring either inadvertently or deliberately, it results mostly from everyday human activities through releases into coastal waters and land. Scores of oil spills are not reported in developing countries and many times, concise efforts are not made to restore the ecosystem to its previous state even when the oil spills are accounted for. Crude oil spills have damaged vulnerable ecosystems across the world. Offshore oil spill is the major cause of concern because of its hazardous impacts on marine life. Conventional countermeasures for oil spill remediation in the aquatic environment include various physical, chemical, thermal, and biological processes. Biological processes involving the use of many native microorganisms in water and soil are preferred because they involve the use of living organisms to carry out remediation of polluted sites, particularly the cleanup of crude oil spills in the detoxification of these environments. Although biological methods are not free of disadvantages, the benefits are quite enormous and these include the fact that they are highly sustainable and at the same time economical.

Chhatre, *et al.,* (1996) carried out a study to examine Bacterial consortia for crude oil spill remediation. Oil spills generate enormous public concern and highlight the need for cost effective and environmentally acceptable mitigation technologies. Physico-chemical methods are not completely effective after a spill. Hence, there is a need for improved and alternative technologies. Bioremediation is the most environmentally sound technology for cleanup. This report intends to determine the potential of a bacterial consortium for degradation of Gulf and Bombay High crude oil. A number of bacteria were isolated from an acclimated semicontinuous reactor fed with crude oil. A four membered consortium was designed that could degrade 70% of the crude oil. A member of consortium produced a biosurfactant, rhamnolipid, that emulsified crude oil efficiently for effective degradation by the other members of consortium. The wide range of hydrocarbon clastic capabilities of the selected members of bacterial consortium leads to the degradation of both aromatic and aliphatic fractions of crude oil in 72 hours.

II. MATERIALS AND METHOD

2.1 Materials

Loamy soil samples were collected from Agbonchia Community in Eleme Local Government Area of Rivers State. Fresh Citrus sinensis (sweet orange) fruits were obtained from local markets and processed to obtain citrus albedo.The apparatus used included conical flasks, measuring cylinders, separator flasks, beakers, filter papers, volumetric flasks, wash bottles with distilled water, thermometer, magnetic stirrer, and Erlenmeyer flasks.Analytical equipment employed comprised a pH meter (WinLab 3005), conductivity/TDS meter (Model 2336), Lamotte colorimeter (Smart 3), Biotech Atomic Absorption Spectrophotometer (Model 1506), electric oven, infrared analyzer, and polarographic membrane electrode.Reagents used included potassium dichromate, silver nitrate solution, sulphuric acid, manganese sulphate, alkaline iodide-azide, ferrous ammonium sulphate, tetrachloroethylene, silica gel, barium chloride, and ferroin indicator.

2.2 Method

A controlled laboratory experiment was conducted to evaluate the effectiveness of citrus albedo in remediating hydrocarbon-contaminated soil. The study examined the effects of two processing methods (sun-dried and room-dried citrus albedo) and three dosage levels (50 g, 100 g, and 150 g).

The experimental design adopted was a Completely Randomized Design (CRD), consisting of two treatment groups and a control. Each treatment involved the application of processed citrus albedo at varying concentrations to contaminated soil samples, while the control sample received no treatment. The impact on pollutant reduction and soil physicochemical properties was then assessed.

2.3.1 Soil Preparation and Containment

As experimental units, 5L plastic containers (buckets) were taken. The soil was collected and characterized beforehand in 1 kg bucket; 500 mL of crude oil was added so as to contaminate the soil. The soil was well blended to make it evenly distributed with the hydrocarbons. To treat a treatment, that treatment amount of citrus albedo (50 grams, 100 grams, or 150 grams) was properly mixed into the soil using a hand to ensure an intimate contact between the organic substance and the soil particles. The citrus albedo was made up in a separate manner as follows:

i. **Sun-Dried Albedo (SDA):** Dried in open sunlight 7 days to brittle, ground and sieved.
ii. **Room-Dried Albedo (RDA):** dried indoors with ambient room temperature (2528 and 1/12 C) of 10 - 12 days to a feasible degree of dryness, and ground and sieved.

The amendments were done on a weight to weight (w/w) basis which was computed against the amount of soil 1/kg taken in individual unit. This was the same as an application rate of about 5%w/w, 10%w/w and 15%w/w of 50g, 100g and 150g, respectively.

2.3.2 Incubation Conditions and Monitoring

The entire treatment units were located in a laboratory working area that was well ventilated and shaded to reduce direct exposures to sunlight and rain falls. Soil moisture content was maintained at approximately 60% of field capacity throughout the 35-day remediation period by regularly adding distilled water to each container, based on gravimetric monitoring.

Soil samples were collected from each treatment group at Day 0 (before amendment application) and subsequently at Day 7, Day 14, Day 21, Day 28 and Day 35. Each sampling was conducted by taking soil from three different depths and locations within the bucket to form a composite sample representative of the entire container.

2.3.3 Parameters for Investigations

The parameters to be analyzed include but not limited to: pH, Temperature, conductivity, Total dissolved solids (TDS), Chloride content, Tubidity, Total Suspended Solids (TSS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD5), Chemical Oxygen Demand (COD), Total Hydrocarbon Content (THC), Sulphate. Heavy metals such as Lead (Pb), Total Iron (Fe).

2.3.4 Experiment: Analysis on the samples.

PH, Electrical conductivity, and Total Dissolved Solids: A Bench top meter was used for pH, Electrical Conductivity and Total Dissolved Solids measurement. The equipment comes with various probes which were first calibrated with certified reference standards such as pH buffer 4.01, 7.01 and 10.01 is for pH calibration, and 12 8ms/cm for conductivity calibration. After calibration, the probes were dipped into each sample and the displayed reading allowed to stabilize before recording. The analytical test methods adopted for these measurements were APHA 4500-H+B, APHA 2510B, APHA 2520 B and APHA 2540C.

2.3.5 Moisture Content

The moisture content of the samples was determined according to standard method described by Ukpaka and Edwin, (2013). 10g of soil sample was weighed into a crucible and heated in an oven at 105°C for 24 hours to dry off water content in the soils. After which, the dried soil samples were cooled in desiccator for 30 minutes. On cooling, the samples were reweighed to obtain a constant weight. To calculate the percentage moisture content, Equation 3.1 was used.

$$MC(\%) = \frac{W_1 - W_2}{W_1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, MC is a moisture contents (%), W_1 is an initial weight of soil sample (g) and W_2 is a weight of dried soil sample (g).

2.3.6Total Organic Carbon

Total Organic Carbon (TOC) was determined using a method described by Ukpaka and Edwin, (2013). 1.0g of crushed fine representative soil samples was weighed in duplicate into 250ml beaker. 10ml of potassium dichromate solution was pipette into beakers and then, rotated gently to completely wet the soil sample, and then, followed by addition of 20ml of concentrated H_2SO_4 using an automatic pipette, directing the stream into the suspension. Thereafter, the beaker was gently rotated to obtain a uniform mixture of soil and reagents, and vigorously rotated for the next one minute, for effective and more complete oxidation, before being allowed to stand for 30 minutes on sheet of asbestos. On settling, 100ml of distilled water was added followed by addition of 3-4 drops of 0.5 ml diphenylamine indictor. The solution was titrated with 0.5N ferrous sulfate solution. The end point was noticed as dull green through turbid blue to brilliant green. The process was repeated on distilled water (blank titration), but without soil to standardize the dichromate. The TOC was calculated using Eq. 3.2.

$$TOC = B - \frac{V - 0.195}{W} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where, V is volume, W is weight of sample and B is a lank.

2.3.7Total Petroleum Hydrocarbon (TPH) Content

This was determined using ASTM (1999) method D3921. Hydrocarbon content was extracted with dichloromethane in an extractor and treated with 2 ml of activated silica gel. The TPH of the representative samples was thendetermined with the aid Gas Chromatography – Flame Ionization Detector (GCFID) Model, HP 5890 Series II, U.S.A. The percentage of TPH removed at any given time was determined using Eq. 3.

$$TPH_r(\%) = \frac{TPH_i - TPH_f}{TPH_i} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, TPH_R is the percentage of TPH removed at a given time, TPH_i is the initial concentration of THP and TPH_f is the concentration of TPH at a given time.

2.3.8Total bacteria count (TBC)

Prepared nutrient agar culture plates were made according to the manufacturer's specification (HIMEDIA) M001-500G, HIMEDIA Laboratories Pvt. LTD Number-400086, India). The culture plates were dried and 0.1ml of the diluted soil samples was placed on it and it was spread using a glass rod spreader to dryness on the plate. This was incubated in an incubator at 37ºC for 24hours and the counting of the bacteria was made on the plate after the bacteria have shown growth. The bacteria that did not grow after 24hours was further allowed in the incubator for another 24hours and readings will be made on them.

3.4 Statistical Analysis

All collected data was subjected to analysis of variance (ANOVA) using Python software. Statistical significance was tested at **$p < 0.05$**, and treatment means will be compared using Duncan's Multiple Range Test (DMRT). The relationship between TPH degradation, microbial activity, and soil parameters was assessed through correlation and regression analysis.

III. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Loamy Soil before and after Pollution

The following table shows the physicochemical characteristics of loamy soil before contamination and after the contamination.

Table 1:Physicochemical Properties of Loamy Soil before and after Pollution

Property	**Normal Loamy Soil (Experimental Values)**	**Crude Oil–Contaminated Loamy Soil**	**Remarks**
Texture	Sand 42%, Silt 38%, Clay 20%	Sand 42%, Silt 38%, Clay 20%	Texture unchanged; contamination affects chemistry, not particle size
pH (Soil Reaction)	6.7	5.5	Becomes more acidic due to hydrocarbon breakdown and organic acid release
Bulk Density	1.32 g/cm³	1.48 g/cm³	Increases due to pore clogging by oil
Porosity	50 %	40 %	Reduced; crude oil coats soil particles and blocks pores
Moisture Content (Field Capacity)	25 %	15 %	Decreases because oil film reduces water infiltration and retention
Organic Matter Content	3.5 %	7.8 %	Appears higher due to petroleum hydrocarbons counted as organic matter
Cation Exchange Capacity (CEC)	18 cmol(+)/kg	10 cmol(+)/kg	Drops as hydrocarbons reduce exchange sites availability
Electrical Conductivity (EC)	1.2 dS/m	3.8 dS/m	Increases; crude oil often contains salts and toxic ions
Available Nitrogen (N)	0.21 %	0.08 %	Reduced; microbial immobilization and toxicity affect N cycling
Available Phosphorus (P)	22 mg/kg	9 mg/kg	Drops; oil contamination limits microbial mineralization of P
Available Potassium (K)	145 mg/kg	72 mg/kg	Decreases; nutrient uptake and cycling disrupted
Heavy Metals (e.g., Pb, Cd, V, Ni)	Trace levels (<0.5 mg/kg)	Elevated (Pb: 3.2, Ni: 2.5 mg/kg)	Crude oil introduces toxic heavy metals

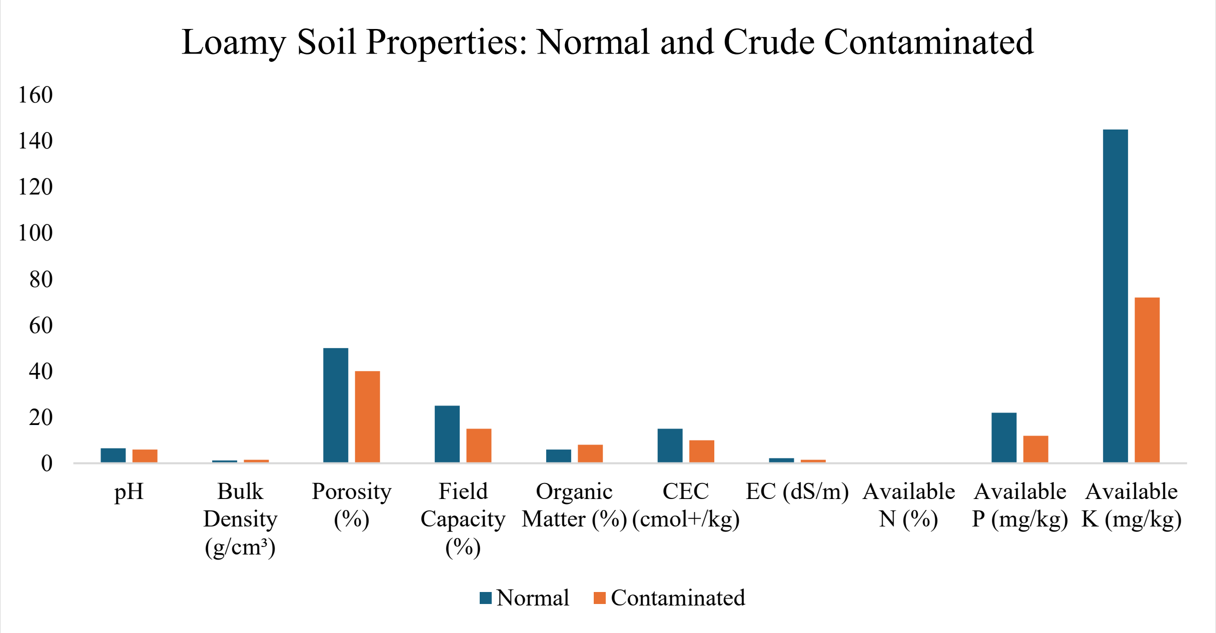

Figure 1: Loamy Soil Properties: Normal and Crude Contaminated

The table 1 above shows the physicochemical characteristics of loamy soil prior to and after being polluted by contamination with crude oil. Loamy soils have been recognised to be the most fertile, due to the balanced quantities of sand, silt and clay, and also favourable physicochemical traits. Loamy soil in natural conditions has a slightly acidic to neutral pH of 6.07.5 that promotes growth of multiple microorganisms and increase in nutrients. This ideal range of 6.7 is the exact level of the experimental determination that the soil is stable and fruitful on the chemical level. Nevertheless, the entrance of crude oil into the soil leads to the pH of the soil being lowered to about 5.5 making the soil acidic. This is in line with the results of Ukpaka, Lezorghia, and Nwosu (2020) who noted that crude oil contamination leads to release of organic acids during hydrocarbon degradation and subsequently leads to acidity of the soil and consequently decreases its fertility potential.

After the contamination of crude oil, the physical properties of the loamy soil change greatly, too. Loamy soil is normally presented with a bulk density of 1.2-1.4g/cm 3 and is porous with 4555 porosity. The conditions are good in terms of aeration and root penetration. The normal soil value of 1.32 -3 g cm -1 used in the experiment and 50 percent porosity shows a healthy soil. Bulk density goes up to 1.48gcm -3 and porosity goes down to 40%. These transformations are explained by the fact that the crude oil is hydrophobic and puts a coating on the soil particles, blocks the pores and forms compaction. Ukpaka et al. (2020) also stated that oil spills cause a rise in the density and decreased porosity of soil, which limits water and air flow necessary to keep plants alive.

Another property of soil that is essential and has been impacted by contaminants is the moisture retention capacity of loamy soil. The soil under its natural state also has about 20-30 per cent moisture at field capacity and this was 25 per cent; this amount is sufficient to support crop growth. However, the soil that contains crude oil has a moisture content of 15 0. The hydrocarbons coated the soil particles and decreased the infiltration and water retention. This fact correlates with Ukpaka et al. (2020), who described that crude oil makes the soil surfaces hydrophobic, which does not allow the effective wetting and greatly -affects seed germination and crop performance.

The level of organic matter is an important measure of soil fertility. Normal loamy soil has 2-5 percent organic matter as a normal and the experiment had a result of 3.5 percent. Once contaminated, the figure increases to 7.8% and the first thing that may cross the mind would earmark an improved fertility. Nonetheless, it has been revealed that the increment is not genuine since crude oil hydrocarbons are counted in chemical analyses as organic matter by Njoku, Akinola, and Taiwo (2008). Hydrocarbons do not positively impact nutrient cycle or soil microbial behavior unlike natural organic matter which is formed as a result of decomposing vegetal and animal remains. On the contrary, they inhibit natural operations and can be even toxic to the soil organisms.

Another characteristic of the loamy soil that has been impacted by the crude oil pollution is the cation exchange capacity (CEC). CEC values of 10-25 cmol(-) kg- are usually ideal values of nutrient retention with the experiment of 18 cmol(-)kg- being reflective of a fertile state. The CEC in soil contaminated with crude oil reduces to 10 -1 cmol kg -1 which is even on the lower end of the acceptable range. Hydrocarbons occupy the active sites of clay and organic matter therefore lessening the capacity of soil to carry vital nutrients. A similar observation has been made by Adenipekun and Lawal (2012) who added that hydrocarbon contamination decreases the CEC of the soil resulting in decreased fertility and crop yield.

Electrical conductivity (EC) gives understanding of the soil salinity. Loamy soil in the normal condition has a low EC level of approximately 1.2 -1 dS m, which is non-saline and not a threat to crops. EC also rises to 3.8 dsm -1 in polluted soil, bordering saline soil conditions. Adoki and Orugbani (2007) put this increment down to salts and trace metals in petroleum products that increase the salinity of soils and are therefore dangerous to the sensitive plants.

The most vital factor that is subjected to the effects of crude oil contamination is the availability of nutrients. At the unpolluted area in the loamy soil, the concentration of nitrogen, phosphorus, and potassium is moderate to adequate with concentrations of 0.21, 22, and 145mgkg and 1 kg respectively. These vitamins play an important role in the development and growth of plants. Following contamination, the values reduce to 0.08-percent nitrogen, 9-mg/kg -1 phosphorus and 72-mg/kg -1 potassium. These drastic-cuts weaken crop performance. Okoh (2006) argued that the effect of hydrocarbons is that they hinder the fixation of nitrogen by bacteria, and Abii and Nwosu (2009) pointed out that under contamination the fixation of phosphorus minerals is inhibited by the inhibition of the activity of the bacteria. Osuji and Nwoye (2007) reported that, there is a great decrease in the potassium content of crude oil contaminated soils, which means that, the cycling of nutrients is interrupted by hydrocarbon contamination.

Lastly, another aspect of degradation attributed to crude oil is heavy metal build-up. Whereas normal loamy soil contains traces of heavy metals, there is a tendency that when the soil is contaminated, the amounts of lead, nickel, vanadium and cadmium are present. These metals are also a danger of toxicity of plants and bioaccumulation in the food chain in the long run due to their introduction to the environment by petroleum hydrocarbons. According to Ogbo and Okhuoya (2011), when contaminated with petroleum, toxic heavy metals accumulate thus reducing the microbial activities and the health of soil.

With these outcomes, it can be argued that the contamination of crude oil overloamy soil turns a fertile and well-structured medium into a degraded system that is acidic with low water retention, depleted nutrient content and a toxic system with hydrocarbons. Without active remediation, these contaminated soils may not be available to agricultural production in the near future since such substances as hydrocarbons, heavy metals, and relevant ecological stressors persist.

4.2 Physiochemical Properties of Citrus Albedo

The table below has been presented with the physiochemical characteristics of both the room-dried and the sun-dried citrus albedo.

Table 2: Physiochemical Properties of Sun-dried and Room-dried Citrus Albedo

Property	Sun-Dried Albedo	Room-Dried Albedo	Remarks
Moisture Content (%)	9.4	12.6	Room drying retains more moisture.
Crude Fiber (%)	25.3	28.4	Room drying better preserves fiber.
Ash Content (%)	3.7	4.1	Slightly higher in room-dried.
Protein (%)	4.3	5.0	Sunlight may cause mild protein denaturation.
Lipids (%)	1.9	2.4	Room drying preserves more oil/lipids.
Carbohydrates (NFE, %)	59.5	56.2	Sun-drying shows higher apparent carbohydrate due to lower moisture.
Pectin Content (mg/g)	15.2	17.8	Shade drying preserves pectin structure.
Total Phenolics (mg GAE/g DW)	9.4	12.6	Sunlight degrades phenolics, higher in room-dried.
Flavonoids (mg QE/g DW)	4.6	6.4	More retained in room drying.
Antioxidant Activity (DPPH % inhibition)	48	64	Higher in room drying, better preservation of bioactives.

The given table in Table 2 indicates the physicochemical characteristics of citrus albedo dried under sun and without sun dehydrating procedures. The sun-dried albedo (9.4 -1% moisture content) also contained less moisture than the room-dried albedo (12.6 -1% moisture content), which experienced a faster process of dehydration due to the direct exposure to the sun. The given observation is said to agree with those of Oluremi et al. (2013), who found out that the direct solar exposure causes significant moisture losses, which in turn causes the values of apparent dry matter to increase.

The room-dried samples contained more crude fibre (28.4) as compared to sun-dried samples (25.3). The same has been observed by Anhwange et al. (2009) who reported that shade drying is more conducive in maintaining fibrous components because it does not cause structural disintegration due to high temperatures and exposure to ultraviolet radiation. Retention of fibre is also nutritionally beneficial because albedo of citrus fruits is a recognised source of dietary fibre in functional foods.

The mineral contents, content of ash, had a similar pattern where the sample dried in a room (4.1 93 acres) showed higher levels compared to the sample dried in the sun (3.7 93 acres), which was supported by Babajide and Oyewole (2013). These writers identified that solar drying has the tendency of destroying mineral retention as a result of volatilisation or leaching.

Better results were obtained using the room-dried albedo which had protein and lipid contents of 5.0 0 percent and 2.4 0 percent respectively than those of the sun-dried samples which were 4.3 0 percent and 1.9 0 percent. These results can be compared to Uchoi et al. (2019), who highlighted that low, ambient drying reduces the oxidative degradation of proteins and lipids, which leads to better nutrition.

The sample which was dried by the sun had high carbohydrate values (59.5 0 -) compared to those dried in rooms (56.2). This growth is not always because of the real growth in carbohydrates per se; it is actually a relative concentration effect brought about by the lower water and protein fractions. This was also reported by Eze and Agbo (2011) when they were comparing fruit matrices in the state of dryness.

Pectin, phenolics and flavonoid bioactive compounds were more retained during room drying. As an example, room dried albedo had 17.8 mg/g -1 pectin, 12.6albedo 6.4mg/g -1 flavonoid, respectively as compared to the sun-dried albedo containing 15.2 mg/g -1, 9.4mg/g -1 and 4.6mg/g -1 pectin, phenolics, flavonoids respectively. These findings are similar to those by Ayala -Zavala et al. (2011), who established that the rate of phenolic and pectin breakdown in citrus tissue during photo-oxidation is increased by sunlight exposure. This is further supported by the higher antioxidant activity of room dried albedo (64 volv in DPPH inhibition) over sun dried albedo (48 volv in DPPH inhibition), as depicted in the research of Londoño-Londoño et al., (2010) who associated drying conditions to existent radical-scavenging activities in citrus by-products.
Summing everything discussed up, though sun drying causes better results with regards to decreasing moisture level and increasing the carbohydrate content, room drying is better when it comes to preserving the structure, minerals, protein, lipids, and, above all, bioactive compounds like flavonoids, phenolics.

IV. CONCLUSION

This study examined the performance evaluation of Albedo of Citrus fruits in bioremediation of Hydrocarbon polluted soil. The study demonstrated that the albedo of citrus fruits possesses remarkable potential as a biostimulant in the bioremediation of hydrocarbon-polluted soil. Both sun-dried and room-dried citrus albedo significantly enhanced total petroleum hydrocarbon (TPH) degradation compared to the untreated control. The research arrives at the conclusion that;

i. Citrus albedo was characterized and its value compared with standard values.
ii. Crude contaminant samples were characterized and its value compared with standard values.
iii. Loamy soil samples were described and their values compared with standard values.

The study provides the following contributions to knowledge:

i. This study identifies citrus fruit albedo, a widely abandoned agricultural waste as a new and promising organic reclamation in the removal of hydrocarbons in soils.
ii. The research findings are supported with empirical evidence suggesting that room drying and sun drying albedo perform differently, importance in maintenance of the remediation performance has been identified to be on processing techniques

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