International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

Efficacy of Periwinkle Shell Ash and Periwinkle Shell Powder in Bioremediation of Crude Oil-Polluted Mangrove Soil

Nduonofit, E. E.*, Awi-Waadu, G. D. B. and Oriakpono, O. E.
Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria

Date of Submission: 05-09-2025 Date of Acceptance: 15-09-2025

ABSTRACT
This study assessed the efficacy of periwinkle shell ash and periwinkle shell powder in bioremediation of crude oil-polluted mangrove soil. The 48-week experiment was conducted using a wooden microcosm (3.75 m2), subdivided into 18 cells (0.25 m), with each containing 20 kg of contaminated soil. Six treatment options were arranged in a completely randomized design. Treatments included unpolluted unamended soil (UPS), polluted unamended soil (PSS), and four amended treatments: PSA (2kg PSA), PSAh (6kg PSA), PSP (2kg PSP), and PSPh (6kg PSP). Soil samples were collected at Week 0 (baseline) and 12-week intervals to evaluate physicochemical properties and total petroleum hydrocarbons (TPH). Total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) were enumerated and characterized using morphological and biochemical analyses. Significant increases ($p<0.05$) in HUB populations were observed in amended soils at Week 24, particularly in treatments PSPh (3.30×108 CFU/g) and PSAh (1.12×108 CFU/g) when compared to PSS (2.60×106 CFU/g) and UPS (1.33×105 CFU/g), indicating enhanced microbial activity. Dominant isolates were Bacillus cereus and Ochrobactrum intermedium. The highest TPH reduction (39.10%) was observed in PSAh while the lowest (8.33%) occurred in PSS. The degradation efficiency trend was as follows: PSAh>PSA>PSPh>PSP>PSS. Amendments also improved soil quality by enhancing pH, total organic nitrogen, and cation exchange capacity, while reducing the concentrations of total organic carbon and conductivity. This study demonstrates that periwinkle shell ash and powder are effective biostimulants for enhancing microbial proliferation and activity; hence, restoring hydrocarbon-impacted mangrove soil. The findings support the integration of agricultural wastes into scalable, ecosystem-based remediation frameworks for degraded tropical coastal environments.

Keywords: Bioremediation, crude oil, mangrove soil, periwinkle shell

I. INTRODUCTION

Crude oil is a complex mixture of hydrocarbons that, when released into the environment, causes widespread ecological and human health concerns [48, 22, 15]. Oil spills occur through well blowouts, tanker accidents, pipeline vandalization, and operational failures, leading to contamination of terrestrial and aquatic co systems[53, 46, 20].The persistence of toxic hydrocarbons such as total petroleum hydrocarbons (TPHs) poses severe risks, as they are resistant to degradation, carcinogenic, and environmentally toxic [3, 47, 25].

In Nigeria, the Niger Delta remains the hotspot of crude oil production with of frequent spills [8, 9]. Mangrove ecosystems in this region are predominantly vulnerable due to their fragile structure and ecological importance as breeding and nursery grounds for fish and crustaceans [23, 54]. Oil contamination alters the physicochemical properties of mangrove soils, disrupts microbial activity and threatens biodiversity, thereby necessitating effective remediation strategies[3, 11].

Bioremediation, which employs microorganisms and nutrient amendments to accelerate hydrocarbon degradation, is widely recognized as a sustainable approach to oil spill management [7, 47]. However, its success often depends on the availability of suitable, low-cost materials that can enhance microbial activity. In

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

Nigeria, diverse animal by-products commonly treated as waste have demonstrated potential for soil amendment and microbial stimulation [35]. Among these, periwinkle shells, abundant in coastal communities, offer dual benefits: they improve soil conditions for microbial degradation and simultaneously provide a sustainable disposal pathway for shell waste that otherwise contributes to environmental pollution, foul odour and siltation of water bodies [53, 35].

Despite their availability, limited research has evaluated the efficacy of periwinkle shells in the remediation of oil-polluted mangroves oils. This study, therefore, investigates their potential as an amendment material for enhancing bioremediation efficiency, with a view to providing an eco-friendly strategy for restoring crude oil-polluted mangrove environments.

II. MATERIALS AND METHODS

2.1 Study Area

Bille Kingdom is an ancient Ijaw clan that is situated in the South Eastern part of Degema Local Government Area of Rivers State, Nigeria. It was founded by Queen Ikpakiaba in the ninth century[36]. Billelies within the mangrove forest region of the Niger Delta, a few feet above the sea level, between latitude 4^o 34' 40" N and longitude 6^o 53' 9" E. The area experiences a tropical climate with two distinct seasons: wet season (April-October) and dry season (November-March), with an annual rainfall of 1862 mm, adequate for all year-round crop production. The inhabitants are mainly engaged in fishing, trading and farming. Bille was chosen as the study area as a result of the high level of environmental pollution from artisanal refining and pipeline vandalization.

2.2 Soil Sample Collection

The study was conducted in situ within a crude oil contaminated mangrove site in Bille Kingdom (4^o 34' 45.07" N, 6^o 53' 17.13" E), which was cordoned off to prevent the impact of tides on the results. Polluted soil was collected at 0 - 15 cm depth with sterilized tools and homogenized following standard procedures [17, 42]. The unpolluted soil was similarly obtained from an area with no history of crude oil contamination.

2.3 Source of Soil Amendment Material

Periwinkle shells used as soil amendment were sourced from Akpan Andem market, Uyo Local Government Area of Akwa Ibom State.

2.4 Preparation of Soil Amendment Material

Periwinkle shells were rinsed with distilled water, sun-dried for five days, and divided into two portions. One portion was calcined, ground and sieved (2 mm) to obtain periwinkle shell ash (PSA), while the other was pulverized and sieved to produce periwinkle shell powder (PSP).

2.5 Experimental Design

A Randomized Complete Block Design (RCBD)with six treatments in triplicate was employed. Treatments included unpolluted soil without amendment (UPS, negative control), polluted soil without amendment (PSS, positive control), and crude oil polluted mangrove soil amended with periwinkle shell ash (PSA) or periwinkle shell powder (PSP) at 10 % (2 kg/20 kg soil) and 30 % (6 kg/20 kg soil) application rates (Table 1).

Table 1. Experimental design for the bioremediation of crude oil polluted mangrove soil

Sample Code	Test Experiment
UPS	20 kg of unpolluted soil + No Amendment Material
PSS	20 kg of polluted soil + No Amendment Material
PSA	20 kg of polluted soil + 2 kg of Periwinkle Shell Ash
PSAh	20 kg of polluted soil + 6 kg of Periwinkle Shell Ash
PSP	20 kg of polluted soil + 2 kg of Periwinkle Shell Powder
PSPh	20 kg of polluted soil + 6 kg of Periwinkle Shell Powder

2.6 Bioremediation Study

The experimental setup was aimed at monitoring the bioremediation of crude oil polluted mangrove soil for forty-eight (48) weeks. Since the mangrove areas experience high and low tides, an area was cordoned off for this study to prevent the impact of the tides. A wooden box was therefore constructed in this area. Before the construction of the box, the area was cleared off with the aid of matchet, spade and hoe. The box covered a total area

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

of 2.20 m^2 (2.16 m × 1.02 m); and was partitioned into eighteen cells. The measurement of each cell was 0.25 m in length, width and height. To prevent treatment drift between adjacent cells, a distance of 0.13 m was maintained between each cell. The quantity of soil in each of the cell was about 20 kg.

Prior to the application of soil amendment materials, soil samples (first set) from each of the cell was collected and analyzed to ascertain the physicochemical properties of the polluted and unpolluted soil. The first set of samples for analysis was taken after the soil in each cell had stabilized for fourteen days and the data obtained served as baseline. After two weeks, 2 kg and 6 kg of periwinkle shell ash and periwinkle shell powder were only added to the cells that contained crude oil polluted mangrove soils with exception to treatment PSS. The amendment materials were thoroughly mixedto ensure homogeneous distribution within the soil.The polluted and unpolluted soils were tilled once in three months. This was carried out in order to enhance the transfer of oxygen into the soil; thereby promoting aerobic degradation of the organic pollutants.

2.7 Sample Collection for Analysis

Soil samples for laboratory analysis were collected on a quarterly basis. The first set of samples were collected 2 weeks after the experimental set up in order to determine the physical and chemical properties of the unpolluted soil and crude oil polluted mangrove soil which served as baseline (Week 0). The second, third, fourth and fifth set of samples were collected at Weeks 12,24, 36 and 48 respectively; after the application of soil amendment materials. 5 g of the composite samples were collected into sterile bottles using sterile spatula for microbial analysis while 200 g of the composite samples were collected in Ziploc bags for physicochemical analysis. The samples were properly labelled on the bottles and Ziploc bags respectively. All samples were taken to the laboratory within two hours after sample collection. The microbiological analysis was carried out in the Microbiology laboratory in University of Port Harcourt while the physicochemical analysis was at Real Tech Research Laboratory in Port Harcourt, both in Rivers State.

2.8 Physicochemical Analyses Soil

Physicochemical properties of soil were determined using standard methods [4]: pH, total organic carbon, total nitrogen, available phosphorus of the soil sample, total phosphorus, cation exchangeable capacity, and electrical conductivity were all determined. Triplicate determinations were made for each assay.

2.9 Total petroleum hydrocarbon (TPH)

Total Petroleum Hydrocarbons (TPH) was extracted from the soil samples and quantified using Gas Chromatograph-Flame according to the methods of ASTDM 3921 and US EPA 8015 analytical protocol (TPI, 2007) as reported by [10] and in accordance with Nigerian requirements of Department of Petroleum Resources (DPR), National Oil Spill Detection Response Agency (NOSDRA) and Federal Ministry of Environment (FMEnv).Samples were kept in a cooler with icepack at 4 °C, labeled appropriately and sent tothe laboratory for analysis. All samples were analyzed in triplicate while ensuring precision and reliability of results through standard quality assurance and control procedures.

2.10 Enumeration and Identification of Bacterial Isolates

10 g of soil sample from the amended options and the control option was introduced into 90 mL of distilled water and shaken vigorously for proper mixing of the sample. A 0.1 mL aliquot of the appropriate dilution of the suspension was inoculated on sterile nutrient agar plates by the spread plate method for total heterotrophic bacteria [32]. The agar plates were incubated at 35 °C for 24 hours after which colony forming units (CFU) per gram of soil samples were calculated. Three replicate samples from each oil-polluted soil were withdrawn every 7 days forthe enumeration of total heterotrophic bacteria (THB). Hydrocarbon utilizing bacteria (HUB) in the soil samples were enumerated by plating on Bushnell Has medium, pH 7.4, using the vapour phase transfer method as described [30]: A filter paper saturated with sterile crude oil was aseptically placed on the inside of the cover of inverted inoculated petri dishes and incubated at 28 °C for 7 days. Distinct colonies of hydrocarbon utilizing bacteria were picked and pure isolates obtained by repeated subculturing on nutrient agar. The bacterial isolates were characterized using microscopic techniques and biochemical tests such as catalase, urease, oxidase, starch hydrolysis, spore forming, H_2S production, motility, citrate utilization and methyl red tests.

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

2.11 Biodegradation Efficiency

Biodegradation efficiency quantifies the decrease in contaminant level overtime due amendment application. This was computed using Equation (1) [35, 51].

$$D = \frac{Co - Ct}{Co} \times 100 \qquad (1)$$

where;D = biodegradation efficiency (%); Co = initial concentration of pollutant in soil sample (mg/kg); Ct = residual concentration of pollutant in soil sample at any time (mg/kg).

2.12 Biostimulation Efficiency

Biostimulation efficiency denotes the effectiveness of amendment materials in improving treatment options. This was calculated using Equation (2) [35, 51].

$$B.E = \frac{D(T) - D(U)}{D(T)} \times 100 \qquad (2)$$

where; B.E = Biostimulation efficiency (%); D(T) = percentage removal of crude oil in the biostimulated soil; D(U) = percentage removal of crude oil in the polluted soil without amendment (PSS).

2.13 Statistical Analysis

The data obtained from this study were subjected to statistical analysis using statistical package for social sciences (SPSS, version 26.0), for quantitative and inferential analysis. A one-way analysis of variance (ANOVA) was adopted for this study. A post-hoc Tukey test was carried out to verify statistically significant differences among individual means at $p \leq 0.05$. The results were presented as Mean ± Standard Deviation.

III. RESULTS

3.1 Physicochemical Properties of Soil Samples

At week 0, the pH of crude oil polluted mangrove soil was acidic while that of the unpolluted soil was alkaline (Figure 1). The data obtained revealed that the mean values of pH increased from Week 0 to Week 48 in unamended polluted soil (PSS) and polluted soil with different amendment materials (PSA, PSAh, PSP, PSPh), with the exception of treatment UPS. The mean values for pH ranged from 4.35 ± 0.03 (Week 0) to 5.52 ± 0.07 (Week 48), 4.53 ± 0.04(Week 0) to 6.85 ± 0.02 (Week 48), 4.47 ± 0.02 (Week 0) to 6.99 ± 0.22 (Week 48), 4.38 ± 0.03 (Week 0) to 6.86 ± 0.04 (Week 48), 4.43 ± 0.01 (Week 0) to 7.13 ± 0.02 (Week 48) for PSS, PSA, PSAh, PSP, and PSPh respectively. There was a significant difference ($p < 0.05$) between the amended treatments and the control (UPS and PSS).

The mean values of total organic carbon revealed that there was a significant difference ($p < 0.05$) between the amended treatments which decreased from 2.88 ± 0.01 % to 2.69 ± 0.00 % in PSA, 2.87 ± 0.00 % to 2.64 ± 0.01 % in PSAh, 2.86 ± 0.00 % to 2.76 ± 0.00 % in PSP, 2.86 ± 0.00 % to 2.74 ± 0.00 % in PSPh (Table 2). There was a slight reduction in the control with mean values of 0.47 ± 0.00 % to 0.46 ± 0.00 % and 2.87 ± 0.00 % to 2.84 ± 0.00 % in UPS and PSS respectively.

The mean values of total organic nitrogen (TON) remained unchanged in UPS (0.04 ± 0.00 %) and PSS (0.25 ± 0.00 %) but increased significantly ($p < 0.05$) in amended soils, reaching 0.28 % in PSA and PSP, 0.29 ± 0.00 % in PSAh, and 0.30 ± 0.00 % in PSPh (Table 3).

The concentration of phosphorus in UPS remained relatively stable throughout the study, with a value of 0.10 ± 0.00 mg/kg (Table 4). For PSS, the mean value of phosphorus were 0.26 ± 0.01 mg/kg, 0.26 ± 0.00 mg/kg, 0.25 ± 0.01 mg/kg, 0.23 ± 0.01 mg/kg, and 0.21 ± 0.01 mg/kg at Week 0, 12, 24, 36, and 48 respectively. For PSA, the mean values ofphosphorus were 0.28 ± 0.01 mg/kg, 0.27 ± 0.01 mg/kg, 0.26 ± 0.01 mg/kg, 0.23 ± 0.01 mg/kg, and 0.21 ± 0.01 mg/kg; while that of PSAh were 0.27 ± 0.02 mg/kg, 0.25 ± 0.01 mg/kg, 0.26 ± 0.01 mg/kg, 0.24 ± 0.01 mg/kg, and 0.23 ± 0.00 mg/kg at Week 0, 12, 24, 36, and 48 respectively. For PSP and PSPh, the mean values of phosphorus were 0.26 ± 0.02 mg/kg, 0.25 ± 0.00 mg/kg, 0.25 ± 0.01 mg/kg, 0.25 ± 0.01 mg/kg, and 0.23 ± 0.01 mg/kg; and 0.27 ± 0.01 mg/kg, 0.25 ± 0.01 mg/kg, 0.25 ± 0.01 mg/kg, 0.24 ± 0.00 mg/kg, and 0.23± 0.01 mg/kg at Week 0, 12, 24, 36, and 48 respectively. The data obtained for phosphorus showed a significant difference ($p < 0.05$) in all amended treatments when compared to the control groups.

The mean values of cation exchangeable capacity (CEC) are presented in Table 5. At week 0, CEC values ranged from 2.18 ± 0.00 meq/100 g in PSAh to 6.55 ± 0.00 meq/100 g in UPS. By week 36, the values ranged from 2.19 ± 0.00 meq/100 g (PSS, PSP) to 6.53 ± 0.00 meq/100 g in UPS. During the 48th week, the values of CEC across the treatments

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

ranged from 2.19 ± 0.00 meq/100 g in PSS to 6.54 ± 0.00 meq/100 g in UPS. The statistical analysis revealed that there was a significant difference ($p < 0.05$) for the different treatment options.

There was variation in electrical conductivity of the crude oil polluted mangrove soil across the treatments as presented in Table 6. At Week 0, electrical conductivity was highest in PSP, with a value of 1154.00 ± 4.00 µs/cm, while the lowest value of 91.00 ± 2.00 µs/cm was observed in UPS. At week 48, PSAh treatment recorded a significant reduction to 978.33 ± 4.62 µs/cm while UPS exhibited a slight reduction to 90.00 ± 0.00 µs/cm. There was a significant mean difference ($p < 0.05$) between the different treatment options and the control groups.

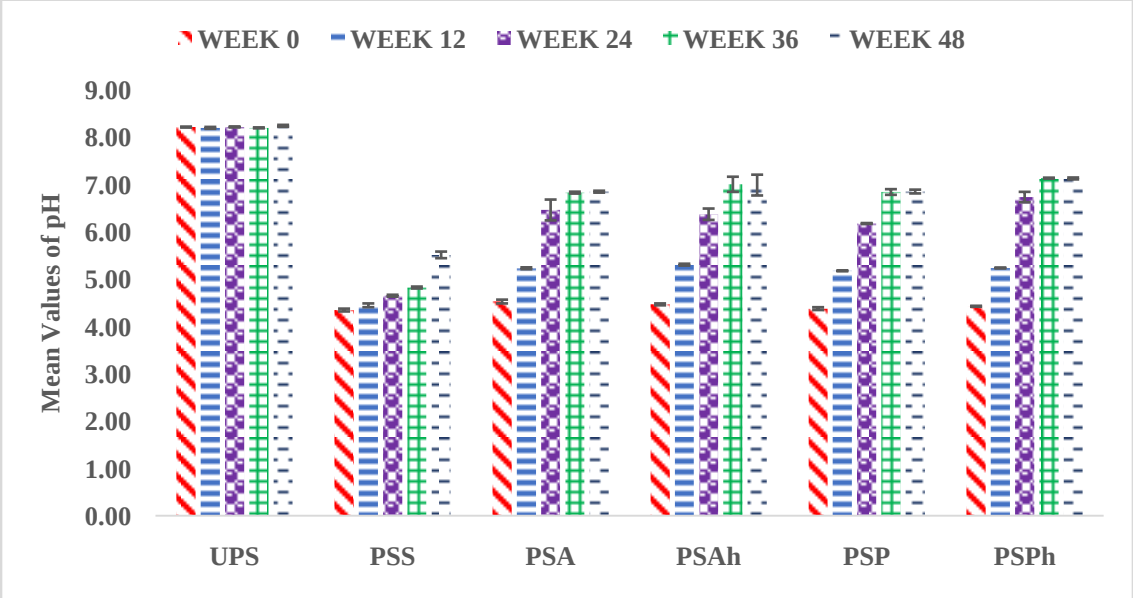

Figure1. Changes in pH during the 48 weeks bioremediation of crude oil polluted mangrove soil

Data are presented as Mean ± SD; different letters on bars per treatment indicate significant difference ($p < 0.05$)
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

Table 2. Changes in total organic carbon (%) during the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Week 0	Week 12	Week 24	Week 36	Week 48
UPS	0.47 ± 0.00^{a}	0.47 ± 0.00^{a}	0.46 ± 0.00^{a}	0.46 ± 0.00^{a}	0.46 ± 0.00^{a}
PSS	2.87 ± 0.00^{cde}	2.87 ± 0.00^{efg}	2.86 ± 0.00^{g}	2.85 ± 0.00^{h}	2.84 ± 0.00^{h}
PSA	2.88 ± 0.01^{efg}	2.86 ± 0.01^{de}	2.74 ± 0.01^{d}	2.69 ± 0.01^{f}	2.69 ± 0.00^{f}
PSAh	2.87 ± 0.00^{def}	2.85 ± 0.01^{df}	2.78 ± 0.01^{e}	2.66 ± 0.02^{f}	2.64 ± 0.01^{f}
PSP	2.86 ± 0.00^{cd}	2.85 ± 0.00^{de}	2.82 ± 0.00^{f}	2.77 ± 0.00^{g}	2.76 ± 0.00^{g}
PSPh	2.86 ± 0.00^{c}	2.81 ± 0.00^{c}	2.79 ± 0.00^{e}	2.75 ± 0.00^{g}	2.74 ± 0.00^{g}

Data are presented as Mean ± SD; different letters on bars per treatment indicate significant difference ($p < 0.05$)
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

Table 3. Changes in total organic nitrogen (%) during the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Week 0	Week 12	Week 24	Week 36	Week 48
UPS	0.04 ± 0.01^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}
PSS	0.25 ± 0.00^{b}	0.25 ± 0.00^{b}	0.25 ± 0.00^{b}	0.25 ± 0.00^{b}	0.25 ± 0.00^{b}
PSA	0.25 ± 0.00^{b}	0.25 ± 0.00^{b}	0.26 ± 0.01^{cd}	0.28 ± 0.01^{c}	0.28 ± 0.01^{c}
PSAh	0.25 ± 0.00^{b}	0.25 ± 0.00^{bc}	0.26 ± 0.00^{c}	0.29 ± 0.00^{d}	0.29 ± 0.00^{d}
PSP	0.25 ± 0.00^{b}	0.26 ± 0.00^{bcd}	0.27 ± 0.00^{de}	0.28 ± 0.00^{c}	0.28 ± 0.00^{c}
PSPh	0.25 ± 0.00^{b}	0.26 ± 0.00^{bcd}	0.29 ± 0.00^{f}	0.30 ± 0.00^{d}	0.30 ± 0.00^{d}

Data are presented as Mean ± SD; different letters on bars per treatment indicate significant difference ($p < 0.05$)
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

Table 4. Changes in phosphorus (mg/kg) during the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Week 0	Week 12	Week 24	Week 36	Week 48
UPS	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}
PSS	0.26 ± 0.01^{bc}	0.26 ± 0.00^{cde}	0.25 ± 0.01^{bcde}	0.23 ± 0.01^{ef}	0.21 ± 0.01^{def}
PSA	0.28 ± 0.01^{bc}	0.27 ± 0.01^{de}	0.26 ± 0.01^{de}	0.23 ± 0.01^{ef}	0.21 ± 0.01^{def}
PSAh	0.27 ± 0.02^{bc}	0.25 ± 0.01^{bcd}	0.26 ± 0.01^{e}	0.24 ± 0.01^{f}	0.23 ± 0.00^{ef}
PSP	0.26 ± 0.02^{bc}	0.25 ± 0.00^{bcd}	0.25 ± 0.01^{bcde}	0.25 ± 0.01^{f}	0.23 ± 0.01^{f}
PSPh	0.27 ± 0.01^{bc}	0.25 ± 0.01^{bcd}	0.25 ± 0.01^{cde}	0.24 ± 0.00^{ef}	0.23 ± 0.01^{ef}

Data are presented as Mean ± SD; different letters on bars per treatment indicate significant difference ($p < 0.05$)
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

Table 5. Changes in cation exchangeable capacity (meq/100 g) during the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Week 0	Week 12	Week 24	Week 36	Week 48
UPS	6.55 ± 0.00^{g}	6.54 ± 0.00^{g}	6.54 ± 0.00^{g}	6.53 ± 0.00^{f}	6.54 ± 0.00^{g}
PSS	2.21 ± 0.00^{c}	2.20 ± 0.00^{c}	2.20 ± 0.00^{bc}	2.19 ± 0.00^{a}	2.19 ± 0.00^{ab}
PSA	2.24 ± 0.00^{f}	2.25 ± 0.00^{f}	2.26 ± 0.00^{f}	2.27 ± 0.00^{d}	2.28 ± 0.00^{e}
PSAh	2.18 ± 0.00^{b}	2.19 ± 0.00^{b}	2.23 ± 0.00^{d}	2.24 ± 0.00^{c}	2.25 ± 0.00^{d}
PSP	2.22 ± 0.00^{d}	2.21 ± 0.00^{cd}	2.20 ± 0.00^{bc}	2.19 ± 0.00^{ab}	2.20 ± 0.00^{bc}
PSPh	2.22 ± 0.00^{d}	2.21 ± 0.00^{d}	2.20 ± 0.00^{c}	2.20 ± 0.00^{b}	2.20 ± 0.00^{c}

Data are presented as Mean ± SD; different letters on bars per treatment indicate significant difference ($p < 0.05$)
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

Table 6. Changes in electrical conductivity (µs/cm) during the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Week 0	Week 12	Week 24	Week 36	Week 48
UPS	91.00 ± 2.00^{a}	91.00 ± 0.00^{a}	91.00 ± 0.00^{a}	91.00 ± 0.00^{a}	90.00 ± 0.00^{a}
PSS	1082.00 ± 4.00^{c}	1082.00 ± 0.00^{c}	1057.00 ± 0.00^{d}	1041.00 ± 0.00^{f}	1038.00 ± 1.00^{f}
PSA	1147.00 ± 2.00^{f}	1115.00 ± 8.19^{d}	1089.67 ± 6.66^{f}	1017.67 ± 6.03^{e}	1014.33 ± 2.08^{e}
PSAh	1127.00 ± 2.00^{e}	1085.00 ± 3.61^{c}	1025.00 ± 2.00^{c}	981.00 ± 6.24^{d}	978.33 ± 4.62^{d}
PSP	1154.00 ± 4.00^{f}	1145.33 ± 3.06^{e}	1123.67 ± 2.52^{g}	1089.33 ± 7.57^{g}	1079.33 ± 1.53^{g}
PSPh	1098.00 ± 1.00^{d}	1087.67 ± 1.53^{c}	1056.33 ± 4.51^{d}	1020.67 ± 2.08^{e}	1017.67 ± 0.58^{e}

Data are presented as Mean ± SD; different letters on bars per treatment indicate significant difference ($p < 0.05$)
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

3.2 Total Petroleum Hydrocarbon

The changes in the concentration of total petroleum hydrocarbon (TPH) are presented in Figure 2. The level of TPH in UPS was below detectable limit. ForPSS, there was a slight reduction in TPH values from 963.59 ± 0.19 mg/kg (Week 0) to 881.30 ± 0.14 mg/kg (Week 48). In treatment PSA and PSAh, the TPH concentration decreased from 1139.46 ± 0.02 mg/kg and 1112.37 ± 0.02 mg/kg in Week 0 to 696.51 ± 2.40 mg/kg and 677.48 ± 0.78 mg/kg in Week 48 respectively. In treatment PSP and PSPh, there was also a significant reduction ($p < 0.05$) in the concentration of TPH from 981.49 ± 0.00 mg/kg and 987.32 ± 0.00 mg/kg in Week 0 to 772.94 ± 0.71 mg/kg and 696.01 ± 0.80 mg/kg in Week 48 respectively. The trend for biodegradation and biostimulation efficiency of TPH is as follows: PSAh (39.10 %; 78.67 %) > PSA (38.87 %; 78.57 %) >PSPh (29.51 %; 71.77 %) > PSP (21.25 %; 60.80 %) > PSS (8.33 %; 0.00 %) (Table 7).

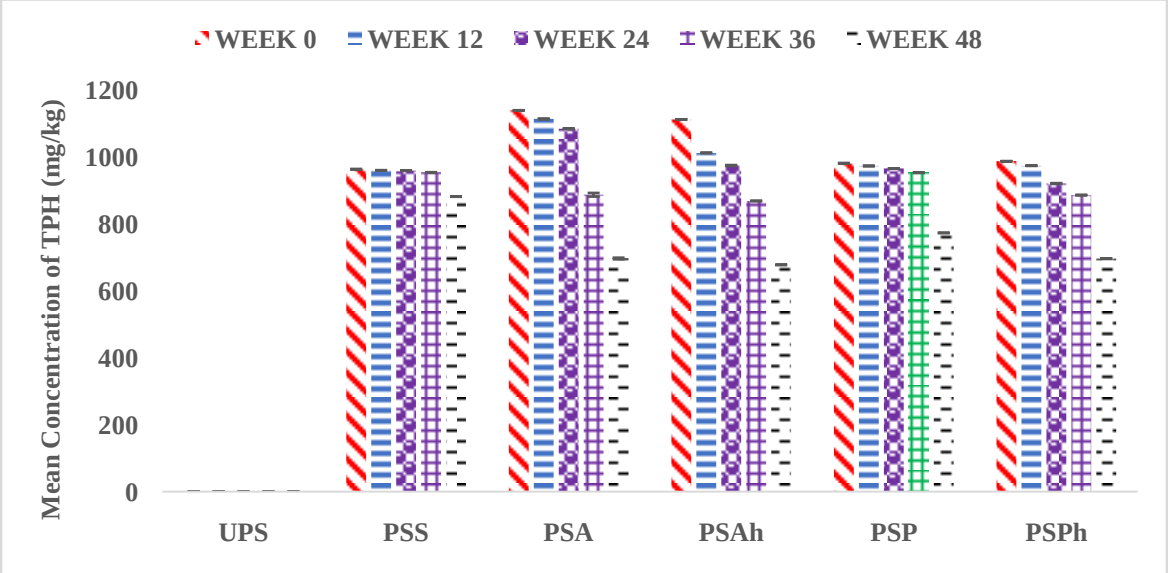

Figure 2. Changes in total petroleum hydrocarbon (mg/kg) during the 48 weeks bioremediation of crude oil polluted mangrove soil

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

Data are presented as Mean ± SD; different letters on bars per treatment indicate significant difference ($p < 0.05$)
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

Table 7. Bioremediation analysis of total petroleum hydrocarbon (TPH) across the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Amount of TPH Remediated (mg/kg)	Overall Biodegradation Efficiency (%)	Biostimulation Efficiency (%)
UPS	-	-	-
PSS	80.29	8.33	-
PSA	442.95	38.87	78.57
PSAh	434.89	39.10	78.67
PSP	208.55	21.25	60.80
PSPh	291.31	29.51	71.77

UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

3.3 Microbial Population During Bioremediation of Crude oil Polluted Mangrove Soil

The population of total heterotrophic bacteria (THB) is presented in Tables 8 and 9. The mean values of THB count for PSA, PSAh, PSP and PSPh were 1.50 × 106 ± 1.00 CFU/g (Week 0) and 2.94 × 108 ± 2.00 CFU/g (Week 48); 3.60 × 106 ± 2.00 CFU/g (Week 0) and 3.88 × 108 ± 1.00 CFU/g (Week 48); 1.20 × 106 ± 1.00 CFU/g (Week 0) and 5.11 × 107 ± 2.00 CFU/g (Week 48); and 2.90 × 106 ± 1.00 CFU/g (Week 0) and 7.21 × 107 ± 2.00 CFU/g (Week 48) respectively.

In UPS, the HUB count decreased from 1.50 × 105 ± 1.00 CFU/g (Week 0) to 1.25 × 104 ± 2.00 CFU/g (Week 48) whereas it increased in PSS from 1.20 ×105 ± 1.00 CFU/g (Week 0) to 7.42 × 106 ± 2.00 CFU/g (Week 48). The mean values of HUB count for PSA, PSAh, PSP, and PSPh were 1.30 × 105 ± 2.00 CFU/g (Week 0) and 8.90 × 107 ± 1.00 CFU/g (week 48); 2.90 × 105 ± 3.00 CFU/g (Week 0) and 1.21 × 108 ± 2.00 CFU/g (week 48); 1.40 × 105 ± 1.00 CFU/g (Week 0) and 2.60 × 107 ± 3.00 CFU/g (Week 48); and 2.10 × 105 ± 1.00 CFU/g (Week 0) and 3.97 × 107 ± 2.00 CFU/g (Week 48) respectively.

Table 8. Changes in Total Heterotrophic Bacteria (CFU/g) during the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Week 0	Week 12	Week 24	Week 36	Week 48
UPS	$1.40 \times 10^{6} \pm 2.00^{b}$	$1.06 \times 10^{6} \pm 1.00^{a}$	$6.98 \times 10^{5} \pm 2.00^{a}$	$7.31 \times 10^{4} \pm 1.00^{a}$	$4.00 \times 10^{4} \pm 2.00^{a}$
PSS	$1.20 \times 10^{6} \pm 1.00^{a}$	$4.69 \times 10^{6} \pm 8.00^{a}$	$6.09 \times 10^{6} \pm 6.00^{ab}$	$1.54 \times 10^{7} \pm 3.00^{b}$	$1.89 \times 10^{7} \pm 2.00^{b}$
PSA	$1.50 \times 10^{6} \pm 1.00^{b}$	$2.00 \times 10^{7} \pm 1.00^{b}$	$2.33 \times 10^{7} \pm 2.00^{bc}$	$2.14 \times 10^{8} \pm 2.00^{h}$	$2.94 \times 10^{8} \pm 2.00^{h}$
PSA_h	$3.60 \times 10^{6} \pm 2.00^{e}$	$1.95 \times 10^{8} \pm 2.00^{g}$	$4.48 \times 10^{8} \pm 1.00^{f}$	$4.24 \times 10^{8} \pm 2.00^{j}$	$3.88 \times 10^{8} \pm 1.00^{i}$
PSP	$1.20 \times 10^{6} \pm 1.00^{a}$	$4.31 \times 10^{7} \pm 2.00^{c}$	$3.50 \times 10^{7} \pm 1.00^{c}$	$4.07 \times 10^{7} \pm 2.00^{c}$	$5.11 \times 10^{7} \pm 2.00^{d}$
PSP_h	$2.90 \times 10^{6} \pm 1.00^{d}$	$7.52 \times 10^{8} \pm 1.00^{i}$	$7.04 \times 10^{8} \pm 1.00^{g}$	$9.96 \times 10^{7} \pm 1.00^{e}$	$7.21 \times 10^{7} \pm 2.00^{e}$
CCB	$1.20 \times 10^{6} \pm 2.00^{a}$	$1.67 \times 10^{8} \pm 2.00^{e}$	$1.33 \times 10^{8} \pm 2.00^{d}$	$1.32 \times 10^{8} \pm 1.00^{f}$	$1.30 \times 10^{8} \pm 3.00^{f}$
CCB_h	$3.80 \times 10^{6} \pm 1.00^{f}$	$3.30 \times 10^{8} \pm 1.00^{h}$	$2.43 \times 10^{8} \pm 1.00^{e}$	$3.67 \times 10^{8} \pm 2.00^{i}$	$2.05 \times 10^{8} \pm 2.00^{g}$
YPC	$1.20 \times 10^{6} \pm 1.00^{a}$	$1.35 \times 10^{8} \pm 2.00^{d}$	$1.17 \times 10^{9} \pm 2.00^{h}$	$5.32 \times 10^{7} \pm 2.00^{d}$	$4.46 \times 10^{7} \pm 1.00^{c}$
YPC_h	$2.30 \times 10^{6} \pm 1.00^{c}$	$1.78 \times 10^{8} \pm 1.00^{f}$	$1.17 \times 10^{9} \pm 1.00^{h}$	$1.70 \times 10^{8} \pm 1.00^{g}$	$2.91 \times 10^{6} \pm 1.00^{a}$

International Journal of Advances in Engineering and Management (IJAEM)
Volume 7, Issue 09 Sept. 2025, pp: 344-357 www.ijaem.net ISSN: 2395-5252

Data are presented as Mean ± SD; different superscript characters in the same column indicate significant difference ($p < 0.05$)

UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

Table 9. Changes in Hydrocarbon Utilizing Bacteria (CFU/g) during the 48 weeks bioremediation of crude oil polluted mangrove soil

Treatment	Week 0	Week 12	Week 24	Week 36	Week 48
UPS	$1.50 \times 10^5 \pm 1.00^{ab}$	$1.48 \times 10^5 \pm 1.00^{a}$	$1.33 \times 10^5 \pm 2.00^{a}$	$1.90 \times 10^4 \pm 1.00^{a}$	$1.25 \times 10^4 \pm 2.00^{a}$
PSS	$1.20 \times 10^5 \pm 1.00^{a}$	$1.80 \times 10^6 \pm 1.00^{b}$	$2.60 \times 10^6 \pm 1.00^{ab}$	$5.83 \times 10^6 \pm 2.00^{b}$	$7.42 \times 10^6 \pm 2.00^{b}$
PSA	$1.30 \times 10^5 \pm 2.00^{a}$	$2.50 \times 10^6 \pm 1.00^{b}$	$5.40 \times 10^6 \pm 2.00^{b}$	$5.90 \times 10^7 \pm 3.00^{g}$	$8.90 \times 10^7 \pm 1.00^{g}$
PSA_h	$2.90 \times 10^5 \pm 3.00^{d}$	$2.90 \times 10^7 \pm 1.00^{e}$	$1.12 \times 10^8 \pm 1.00^{f}$	$1.42 \times 10^8 \pm 2.00^{j}$	$1.21 \times 10^8 \pm 2.00^{h}$
PSP	$1.40 \times 10^5 \pm 1.00^{a}$	$1.50 \times 10^7 \pm 5.00^{c}$	$1.49 \times 10^7 \pm 2.00^{c}$	$1.70 \times 10^7 \pm 2.00^{c}$	$2.60 \times 10^7 \pm 3.00^{d}$
PSP_h	$2.10 \times 10^5 \pm 1.00^{c}$	$2.90 \times 10^8 \pm 1.00^{i}$	$3.30 \times 10^8 \pm 2.00^{g}$	$3.90 \times 10^7 \pm 1.00^{f}$	$3.97 \times 10^7 \pm 2.00^{e}$
CCB	$1.80 \times 10^5 \pm 3.00^{bc}$	$2.70 \times 10^7 \pm 1.00^{d}$	$2.90 \times 10^7 \pm 1.00^{d}$	$3.10 \times 10^7 \pm 1.00^{e}$	$3.80 \times 10^7 \pm 1.00^{e}$
CCB_h	$2.60 \times 10^5 \pm 3.00^{d}$	$5.68 \times 10^7 \pm 1.00^{g}$	$7.11 \times 10^7 \pm 4.00^{e}$	$9.38 \times 10^7 \pm 1.00^{h}$	$8.42 \times 10^7 \pm 3.00^{f}$
YPC	$1.20 \times 10^5 \pm 4.01^{ab}$	$3.53 \times 10^7 \pm 4.00^{f}$	$6.10 \times 10^8 \pm 3.00^{h}$	$2.30 \times 10^7 \pm 3.00^{d}$	$1.90 \times 10^7 \pm 1.00^{c}$
YPC_h	$1.80 \times 10^5 \pm 1.00^{bc}$	$5.84 \times 10^7 \pm 1.00^{h}$	$8.61 \times 10^8 \pm 2.00^{i}$	$1.21 \times 10^8 \pm 2.00^{i}$	$1.81 \times 10^6 \pm 1.00^{a}$

Data are presented as Mean ± SD; different superscript characters in the same column indicate significant difference ($p < 0.05$)

UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

3.4Biochemical and molecular identification of hydrocarbon utilizing bacterial isolates

The bacterial genera isolated form crude oil polluted mangrove soil at week 0 in all the amended treatments were Bacillus species. At week 24, the bacterial isolates identified in treatments PSS, PSP, and PSPh, were Bacillus species, while Ochrobactrum species were identified in PSA and PSAh(Table 10).

The molecular identification of hydrocarbon utilizing bacterial isolates from crude oil polluted soil at week 48 revealed Bacillus cereus in PSS, PSP, PSPh, and Ochrobactrum inter medium in PSA and PSAh.

Table 10. Biochemical characteristics of hydrocarbon utilizing bacterial isolates from crude oil polluted soil at week 48

Treatment	Morphology	Gram stain	Motility	Citrate	Catalase	Indole	Methyl red	Voges Proskauer	Starch	H_2S production	Oxidase	Glucose	Lactose	Mannitol	Sucrose	Probable Organism
UPS	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp
PSS	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp
PSA	R	GN	+	+	+	-	-	-	+	-	+	-	+	NA	NA	Ochrobactrumsp
PSA_h	R	GN	+	+	+	-	-	-	+	-	+	-	+	NA	NA	Ochrobactrumsp
PSP	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp
PSP_h	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp
CCB	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp
CCB_h	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp
YPC	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp
YPC_h	R	GP	+	+	+	-	+	+	+	-	-	+	+	+	+	Bacillussp

R - Rod; **GN** - Gram negative; **GP** - Gram positive; **NA** - Not available
UPS- Unpolluted soil without amendment; **PSS-** Polluted soil without amendment; **PSA-** Polluted soil + 2 kg Periwinkle Shell Ash; **PSAh-** Polluted soil + 6 kg Periwinkle Shell Ash; **PSP-** Polluted soil + 2 kg Periwinkle Shell Powder; **PSPh-** Polluted soil + 6 kg Periwinkle Shell Powder

IV. DISCUSSION

Baseline analysis confirmed that crude oil pollution reduced soil pH to acidic levels, while the unpolluted control remained alkaline. Following amendment, soil pH values shifted toward neutrality, conditions known to enhance microbial growth and hydrocarbon degradation [14, 26, 27]. Neutral to slightly alkaline conditions (pH 6.5–8.0) are optimal for hydrocarbon-degrading consortia [5]. The alkalinity contributed by periwinkle shell ash and powder likely buffered the polluted soils, consistent with earlier reports that alkaline amendments improve microbial colonization and contaminant breakdown [16, 50, 41].

The reduction in total organic carbon (TOC) in amended soils suggests efficient microbial assimilation of hydrocarbons as primary carbon sources [6]. This corroborates reports that organic amendments stimulate hydrocarbon mineralization and pollutant dispersion, thereby increasing microbial accessibility [49, 2]. Sustained TOC depletion across sampling intervals reflects progressive contaminant transformation.

Nitrogen availability is a critical determinant of hydrocarbon biodegradation due to its role in balancing the C:N ratio [5]. Polluted unamended soils showed stable but low total organic nitrogen (TON), indicating impaired nitrogen cycling likely due to hydrocarbon toxicity [57]. Conversely, amended soils exhibited progressive increases in TON, consistent with nitrogen enrichment through microbial fixation and organic nutrient release [37, 21]. Enhanced TON availability supported higher microbial biomass and accelerated contaminant breakdown.

Phosphorus concentrations declined steadily in polluted unamended soils, reflecting immobilization and reduced bioavailability under hydrocarbon stress [13]. By contrast, periwinkle shell ash maintained relatively stable phosphorus levels, likely due to the presence of calcium phosphate, which acts as a slow-release nutrient source [18]. This agrees with studies reporting that phosphorus amendments sustain microbial metabolism and hydrocarbon biodegradation [40].

Cation exchange capacity declined in unamended polluted soil, indicating disruption of nutrient retention and ion exchange capacity by hydrocarbons [41]. Amendments improved CEC, reflecting their role as cation reservoirs and enhancers of nutrient bioavailability, thereby facilitating microbial proliferation [40, 2].

Electrical conductivity exceeded 1000 µs/cm in polluted soils, reflecting saline/brackish mangrove conditions. A progressive decline in EC across amended treatments indicated microbial uptake of dissolved ions, which serve as micronutrients for growth [1, 16]. Similar declines in conductivity during remediation have been attributed to ionic assimilation during active biodegradation [12].

Total petroleum hydrocarbons (TPH) decreased significantly in amended soils, particularly between 24 - 48 weeks, confirming enhanced microbial degradation facilitated by improved soil physicochemical conditions [2].

Unamended controls exhibited only minor TPH declines, attributable to abiotic processes such as volatilization, photodegradation, and adsorption [56, 39]. These results underscore the role of periwinkle shell amendments in sustaining biotic degradation pathways.

Microbial community responses further validate amendment efficacy. Amended soils exhibited exponential increases in total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) compared to unamended controls. This trend aligns with prior evidence that nutrient inputs enhance microbial diversity and hydrocarbon metabolism in contaminated soils [24, 42, 55]. Elevated bacterial counts, particularly in periwinkle shell-amended treatments, suggest amendments enhanced hydrocarbon bioavailability by reducing hydrophobicity and improving mass transfer [38].

Isolates identified as Bacillus cereus and Ochrobactrum intermedium demonstrated hydrocarbon-degrading potential, consistent with earlier studies reporting adaptive consortia that sequentially metabolize hydrocarbon fractions during long-term exposure [33, 29]. Their proliferation in amended soils highlights the role of indigenous microbial consortia in sustained hydrocarbon attenuation.

V. CONCLUSION

The results indicate that periwinkle shell ash andperiwinkle shell powder enhanced soil buffering capacity, nutrient availability, microbial activity and hydrocarbon degradation efficiency. These findings position periwinkle shells as low-cost, environmentally sustainable bioremediation agents for restoring crude oil-polluted mangrove ecosystems.

REFERENCES

[1]. Abu, G. O. and Akomah, O. N. (2008). A laboratory assessment of anaerobic biodegradation of petroleum hydrocarbons in a typical Niger Delta Wetland. Global Journal of Pure and Applied Sciences, 14(1): 97-102.

[2]. Adeniji, A. O., Okaiyeto, K., George, M. J., Tanor, E. B., Semerjian, L. and Okoh, A. I. (2023). A systematic assessment of research trends on polycyclic aromatic hydrocarbons in different environmental compartments using bibliometric parameters. Environmental Geochemistry and Health, 45(5), 1289-1309.

[3]. Alvarez, V. M., Marques, J. M., Korenblum, E., and Seldin, L. (2011). Comparative bioremediation of crude oil-amended tropical soil microcosms by natural attenuation, bioaugmentation or bioenrichment. Applied and Environmental Soil Science, 7(2): 33-48.

[4]. APHA (2008). American Public Health Association, Standard methods for the examination of soil, 21st edition, Washington DC.

[5]. Atlas, R. M. (1981). Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbiological Review, 45: 180-209.

[6]. Benson, D. M., Ochekwu, E. B. and Tanee, F. G. (2016). Enhancement of crude oil polluted soil by applying single and combined cow-dung and hydrogen peroxide as remediating agents. Journal of Applied Sciences and Environmental Management, 20(4), 1137-1145.

[7]. Bidoia, E. D., Montagnolli, R. N. and Lopes, P. R. (2010). Microbial biodegradation potential of hydrocarbons evaluated by colorimetric technique: A case study. Applied Microbiology and Biotechnology, 7: 1277-1288.

[8]. Chete, L. N., Adeoti, J. O., Adeyinka, F. M. and Ogundele, O. (2012). Industrial development and growth in Nigeria: Lessons and challenges. Nigerian Institute of Social andEconomic Research, Ibadan learning to compete; work paper 8.

[9]. Chijioke-Osuji, C. C., Ibegbulam, P. N. and Belford, E. J. (2014). Biodegradation of crude oil polluted soil by co-composting with agricultural wastes and inorganic fertilizer. Journal of Natural Sciences Research, 4(6): 28-39.

[10]. Chikere, C. B., Okpokwasili, G. C. andChikere, B. O. (2009). Bacterial diversity in a tropical crude oilpolluted soil undergoing bioremediation.African Journal of Biotechnology, 8(11): 2535-2540.

[11]. Costa, A. S.,Romao, L., Araújo, B., Lucas, S.,Maciel, S., Wisniewski, A. and Alexandre, M. (2012). Environmental strategies to remove volatile aromatic fractions (BTEX) from petroleum industry wastewater using biomass. Bioresource Technology, 105: 31-39.

[12]. Devatha, C. P., Vishal, A. and Rao, J. (2019). Investigation of physical and chemical characteristics on soil due to crude oil contamination and its remediation. Applied Water Science, 9: 1-10.

[13]. Dhanya, M. S. and Kalia, A. (2019). Bioremediation: an eco-friendly cleanup strategy for polyaromatic hydrocarbons from petroleum industry waste. In Bioremediation of Industrial Waste for Environmental Safety: Volume I: Industrial Waste and Its Management (pp. 399-436). Singapore: Springer Singapore.

[14]. Dibble, J. T. and Bartha, R. (1979). Effects of environmental parameters on the biodegradation of oil spillages. Applied Environmental Microbiology, 37: 72-78.

[15]. Edemhanria, L. and Osubor, C. (2022). Optimization of bioremediation enhancement factors in an aged crude oil polluted soil. Jordan Journal of Biological Sciences, 15(2): 289-294.

[16]. Ezekoye, C. C., Amakoromo, E. R. and Ibiene, A. A. (2015). Bioremediation of hydrocarbon polluted mangrove swamp soil

from the Niger Delta using organic and inorganic nutrients. British Biotechnology Journal, 6(2): 62-78.

[17]. Eziuzor, C. S. and Okpokwasili, G. C. (2009). Bioremediation of hydrocarbon contaminated mangrove soil in a bioreactor. Nigerian Journal of Microbiology, 23(1): 1777-1791.

[18]. Hamzah, A. H. P., Heryadi, D. Y., Judijanto, L., Pramono, S. A. and Lestari, N. C. (2024). Production-optimization of biosurfactant from mangrove sediment bacteria using media salinity, differences in carbon source concentration, and pH levels. Journal of Global Innovations in Agricultural Sciences, 12, 391-398.

[19]. Hesnawi, R. M. and Mogadami, F. S. (2013). Bioremediation of Libyan crude oil-contaminated soil under mesophilic and thermophilic conditions. APCBEE Proc., 5: 82-87.

[20]. Ikhumetse, A. A., Abioye, O. P., Ijah, U. J. and Bankole, M. T. (2022). A critical review of oil spills in the Niger Delta aquatic environment: causes, impacts and bioremediation assessment. Environmental Monitoring and Assessment, 194: 816.

[21]. Ikuesen, F. A., Boboye, B. E. and Adetuyi, F. C. (2016). Comparative bioremediation of crude oil contaminated soil samples using activated soil and activated cow dung. Sky Journal of Microbiology Research, 4(4): 21-30.

[22]. Ite, A. E., Harry, T. A., Obadimu, C. O., Asuaiko, E. R. and Inim, I. J. (2018). Petroleum hydrocarbon contamination of surface water and groundwater in the Niger Delta region of Nigeria. Journal of EnvironmentalPollution and Human Health, 6(2): 51-61.

[23]. Kadafa, A. A. (2012). Environmental impacts of oil exploration and exploitation in the Niger Delta of Nigeria. Global Journal of Science Frontier Research Environmentand Earth Sciences, 12(3): 19-28.

[24]. Margesin, R. and Schinner, F. (2001). Bioremediation (natural attenuation and biostimulation) of diesel oil-contaminated soil in an alpine glacier skiing area. Applied and Environmental Microbiology, 67(7): 3127-3133.

[25]. Musa, O. I., Ijah, U. J., Abioye, O. P. and Adebola, M. O. (2021). Microbial determination of hydrocarbon polluted soil in some parts of Niger State, Nigeria. Biosciences and Bioengineering, 6(3): 20-27.

[26]. Nazir, R., Farooq, S., Mudasir, S. and Ali, U. (2015). Factors affecting bioremediation. Journal of Research and Development, 15: 102-109.

[27]. Neina, D. (2019). The role of soil pH in plant nutrition and soil remediation. Applied and Environmental Soil Science, 1-9.

[28]. Nelson, D. W. and Sommers, L. E. (1975). A rapid and accurate method for estimating organic carbon in soil. Proceedings of Seminar on the Petroleum Industry and the Nigerian Environment. NNPC/FMW and H, Warri, Nigeria.164-170.

[29]. Nnabuife, O. O., Ogbonna, J. C., Anyanwu, C., Ike, A. C., Eze, C. N. and Enemuor, S. C. (2022). Mixed bacterial consortium can hamper the efficient degradation of crude oil hydrocarbons. Archives of Microbiology, 204(6): 306.

[30]. Nwogu, T. P., Azubuike,C. C. and Ogugbue, C. J. (2015). Enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with Capraaegagrushircus(goat) manure. Biotechnology Research International, 22-29.

[31]. Obasi, N. A., Eze, E., Anyanwu, D. I. and Okorie, U. C. (2013). Effects of organic manures on the physicochemical properties of crude oil polluted soils. African Journal of Biochemistry Research, 7(6): 67-75.

[32]. Odokuma, L. O. and Ibor, M. N. (2002). Nitrogen fixing bacteria enhanced bioremediation of crude oil polluted soil. Global Journal for Pure and Applied Science, 8(4): 455-468.

[33]. Odokuma, L. O. and Dickson, A. A. (2003). Bioremediation of a crude oil polluted tropical rain forest soil. Global Journal of Environmental Sciences, 2(1): 29-40.

[34]. Oelkers, E. H. and Valsami-Jones, E. (2008). Phosphate mineral reactivity and global sustainability. Element., 4: 83-87.

[35]. Ofoegbu, R. U., Momoh, Y. O. and Nwaogazie, I. L. (2014). Bioremediation of crude oil contaminated soil using organic

and inorganic fertilizers. Journal of Petroleum and Environmental Biotechnology, 6: 1-6.

[36]. Okafor, N. C. (2016). Empowered rural women cooperatives: A sustainable strategy for rural development and change in the Niger Delta region of Nigeria (Master's thesis). Swedish University of Agricultural Sciences.

[37]. Okoh, A. A. and Trejo-Hernandez, M. R, (2006). Remediation of petroleum hydrocarbon polluted systems: exploiting the bioremediation strategies. African Journal of Biotechnology, 5(25): 2520.

[38]. Okonkwo, C. J., Liu, N., Li, J. and Ahmed, A. (2022). Experimental thawing events enhance petroleum hydrocarbons attenuation and enzymatic activities in polluted temperate soils. International Journal of Environmental Science and Technology, 1-12.

[39]. Onuoha, S. C. (2013). Stimulated biodegradation of spent lubricating motor oil in soil amended with animal droppings. Journal of National Science Research, 3(12): 106-116.

[40]. Onwosi, C. O., Nwankwegu, A. S., Enebechi, C. K., Odimba, J. N., Nwuche, C. O. and Igbokwe, V. C. (2018). Bioremediation of Soil Contaminated with Diesel Using Inorganic Nitrogen Sources: Incorporating n th-Order Algorithm in the Evaluation of Process Kinetics. Soil and Sediment Contamination: An International Journal, 27(1), 60-78.

[41]. Oriapkono, O. E., Adetayo, O. and Oriakpono, I. O. (2021). Biostimulation potentials sawdust and cow blood on a spent engine oil polluted soil. International Science and Technology Journal of Namibia, 14: 161-172.

[42]. Orji F. A., Ibiene, A. A. and Dike, E. N. (2012). Laboratory scale bioremediation of petroleum hydrocarbon-polluted mangrove swamp in the Niger Delta using cow dung. Malaysian Journal of Microbiology, 8(4): 219-228.

[43]. Osodeke, V. E. and Osondu, N. E. (2006). Phosphorus distribution along a toposequence of coastal plain sand parent material in South-Eastern Nigeria. Agricultural Journal, 13, 167-171.

[44]. Othaman, N. N., Isa, M. N., Ismail, R. C., Ahmad, M. I. and Hui, C. K. (2020). Factors that affect soil electrical conductivity (EC) based system for smart farming application. In AIP conference proceedings, 2203(1). AIP Publishing.

[45]. Romanus, A. A., Ekundayo, A. O., Aigere, S. P. and Okwu, G. I. (2015). Bacterial degradation of petroleum hydrocarbons in crude oil polluted soil amended with cassava peels. American Journal of Research Communication, 3(7): 99-118.

[46]. Safi, J., Awad, Y. and El-Nahhal, Y. (2014). Bioremediation of diurin in soil and water by cyanobacterial mat. American Journal of Plant Sciences, 5: 1081-1089.

[47]. Sajna, K. V., Sukumaran, R. K., Gottumukkala, L. D. and Pandey, A. (2015). Crude oil biodegradation aided by biosurfactants from Pseudozyma sp. NII 08165 or its culture broth. Bioresource Technology, 191: 131-139.

[48]. Sam, K., Coulon, F. and Prpich, G. (2017). Management of petroleum hydrocarbon contaminated sites in Nigeria: Current challenges and future direction. Land Use Policy, 64: 133-144.

[49]. Udotong, I. R., Uko, M. P. and Udotong, J. I. R. (2015). Microbial diversity of a remote aviation fuel contaminated sediment of a lentic ecosystem in Ibeno, Nigeria. Journal of Environmental & Analytical Toxicology, 5(06).

[50]. Ughala, E., Ogugbue, C. J. and Okpokwasili, G. C. (2019). Enhanced remediation of a long-term crude oil polluted soil using soya bean and poultry residues. International Research Journal of Public and Environmental Health, 6(8): 210-218.

[51]. Ugwoha, E., Amah, V. E. and Oweh, G. O. (2020). Bioremediation of crude oil contaminated soil using pig droppings and bone char. Journal of Advances in Biology and Biotechnology, 23(8): 13-24.

[52]. Ugwoha, E., Amah, V. E. and Oweh, G. O. (2020). Bioremediation of crude oil contaminated soil using pig droppings and bone char. Journal of Advances in Biology and Biotechnology, 23(8): 13-24.

[53]. Ukpaka, C. P. (2011). Modelling the prediction of biokinetics of dissolved oxygen for wet season degradation of petroleum hydrocarbon in pond system. International Journal of Pharmaceutical Research, 2(3): 1-27.

[54]. Uku, P. E. (2024). Modelling for the phytoremediation of crude oil polluted soil using cassava and yam waste peels. Chemistry International, 10(1): 1-9.

[55]. Umanu, G. and Babade, M. F. (2013). Biological degradation of kerosene in soil amended with poultry droppings. International Journal of Advanced Biological Research, 3(2): 254-259.

[56]. Williams, J. O. and Amaechi, V. V. (2017). Bioremediation of hydrocarbon contaminated soil using organic wastes as amendment. Current Studies in Comparative Education, Science and Technology, 4(2): 89-99.

[57]. Zafra, G., Absalon, A. E., Cuevas, M. C. and Cortes-Espinosa, D. V. (2014). Isolation and selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil contaminated soils. Water, Air and Soil pollution, 225: 1826.