International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 11 Issue 2, February 2024

ISSN: 2394-4404

Bioremediation Of Crude Oil Polluted Wet And Dry Soils Using *Pseudomonas Sp.*

Woyengibunugha Ere
Department of Agricultural and Environmental Engineering,
Niger Delta University, Wilberforce Island, Bayelsa State

Sarah A. Nwinee
Science Laboratory Technology (SLT) Department,
Petroleum Training Institute, Effurun, Nigeria

Abstract: This study investigated the bioremediation of wet and dry soils polluted with crude oil using the indigenous bacterium Pseudomonas sp. Physicochemical parameters like pH, EC, TOC, nitrogen, phosphorus, potassium, moisture content, total heterotrophic bacteria (THB) and Total petroleum hydrocarbon (TPH) degradation were analyzed in the treated wet and dry soil samples over 84 days and compared to untreated control soil samples. Crude oil pollution has immediate effect on the soils as total organic carbon (TOC), electrical conductivity (EC) and moisture in the soils were the increased while pH, nitrogen and phosphorus levels decreased from their initial state in the soils. The levels of the physicochemical parameters obtained within the 84 days period under natural attenuation process were not close to the initial values recorded before pollution. On the other hand, the samples with Pseudomonas sp. treatment boosted the nutrients and microbial proliferation, thereby improving the properties of soils. The optimal TPH degradation efficiency was 96.97% for the wet soil and 95.1% for the dry soil, while 47.9% and 41.38% respectively were recorded in natural processes. The findings therefore, established that the inoculation of Pseudomonas sp. in the crude oil polluted soils significantly improved the bioremediation process with capacity to restore the properties of the soils. Hence, Pseudomonas sp. is recommended as promising microbial amendment for cleanup of oil polluted lands, particularly in crude oil impacted areas of the Niger Delta region of Nigeria.

Keywords: Bioremediation, TPH removal, Pseudomonas sp., Wet soil, Dry soil

I. INTRODUCTION

Environmental pollution, including soil pollution by petroleum hydrocarbons, poses serious environmental issues globally. Several factors can be attributed to pollution by petroleum hydrocarbons. Million metric tons of petroleum hydrocarbons are released into the biosphere each year, through exploration, transportation accidents, and improper disposal (Varjani & Upasani, 2017). As complex mixtures, crude oil pollutants are highly recalcitrant and application of some conventional physico-chemical methods as cleanup agents are not usually cost-effective (Das & Chandran, 2011). Additionally, such abiotic approaches can further endanger the environment (Galdames *et al*., 2017).

Conventional remediation via excavation and thermal/chemical treatments are costly and potentially damaging (Borah & Yadav, 2016). Thus, bioremediation utilizing native and introduced hydrocarbon-degrading microbes has emerged as a sustainable alternative (Das & Chandran, 2011). Specialized soil bacteria have evolved to metabolize crude oil components as sole carbon sources (Akpe *et al*., 2015; Al-Hawash *et al*., 2018). Notable among these is *Pseudomonas*, renowned for its metabolic versatility in this role (Umeojiakor *et al*., 2019). Species including *P. aeruginosa*, *P. fluorescence* and *P. putida* produce surface-active biosurfactants, emulsan polymers and co-metabolic enzymes that help solubilize, emulsify and ultimately mineralize complex petroleum residues (Ebadi *et al*., 2017; Bertollo *et al*., 2018).

Naturally-occurring biodegradation relies on intrinsic microbial activity but proceeds slowly (Ere & Amagbo, 2019). Biostimulation or bioaugmentation involving hydrocarbon-degrading agents have, however, demonstrated accelerated degradation rates compared to intrinsic processes alone (Wang *et al*., 2019). Further, physicochemical characteristics of polluted media can control bioremediation efficacy. Properties such as moisture, nutrient status, pH, redox potential and indigenous microbial population dynamics impact hydrocarbon catabolism significantly (Umeda *et al*., 2017; Ere *et al*., 2020).

The present study investigated the bioremediation potential of an indigenous soil *Pseudomonas sp.* isolate in soils artificially contaminated with crude oil. Wet and dry soils were contaminated, inoculated with *Pseudomonas sp.* and monitored over 12 weeks (84 days) under laboratory setups. Untreated soil samples were used as controls to compare degradation potency of of *Pseudomonas sp.* augmented bioremediation over natural attenuation. Changes in various soil properties, microbial indices and total petroleum hydrocarbon depletion rates were monitored over the periods. This is aimed to assess the bioremediation effectiveness of *Pseudomonas sp.* for possible field application at real impacted sites. Such application-oriented efficacy assessments are critical to develop, scale-up and validate promising bioremediation agents and strategies.

II. MATERIALS AND METHODS

A. SOIL CONTAMINATION AND TREATMENT

Wet and dry soils were collected from Igbedi Community and transported to the laboratory for experimental studies. The soil were divided into four and weighed to 5kg each. The weighed soil samples were added into four different containers. Two of the containers received wet soils while the remaining two received dry soil. All soil samples were artificially contaminated with 340 ml of crude oil and left undisturbed for 72 hours. After 72 hours, 50ml *Pseudomonas sp*. was added into one sample each, containing wet and dry soil, while the remaining samples were used as control (without treatment agent).

The initial average TPH concentrations recorded in the wet and dry soil samples after being polluted with crude oil was 35981.09±11.74 mg/kg and 32563.39±6.18 mg/kg, respectively. The prepared samples were allowed to degrade under room temperature over 84 days period.

B. SOIL PHYSICOCHEMICAL AND BACTERIA ANALYSIS

The physicochemical parameters investigated include pH, electrical conductivity (EC), total organic carbon (TOC), nitrogen (N), phosphorus (P), potassium (K), moisture content (MC) and total hydrocarbon degrading bacteria (THB) were determined before and after the pollution. The physicochemical analyses were conducted according the methods described in a previous study by Ere et al. (2020).

C. TPH DEGRADATION ANALYSIS

Total petroleum hydrocarbon (TPH) content was determined before, during and after the pollution. Representative samples were collected every 14 day interval to analyse the various parameters. The TPH concentrations in the treated and control soils were analysed using gas chromatography according to ASTM D7066-04 method (ASTM, 2015). Extracted hydrocarbon content was treated with 2 ml of activated silica gel. The TPH of the representative samples was using Gas Chromatography – Flame Ionization Detector (GCFID) Model, HP 5890 Series II, U.S.A. The percentage of TPH was determined using the formula:

$$TPH_R(\%) = \frac{TPH_i - TPH_t}{TPH_i} \times 100\% \qquad (1)$$

Where, TPH_R is the percentage of TPH removed at a given time (%), TPH_i and TPH_t are the initial and instantaneous concentration of TPH (mg/kg).

III. RESULTS AND DISCUSSION

The values of the tested physicochemical parameters of wet and dry soils before and after crude oil pollution, as well as the values recorded after 84 days of treatment are presented in Tables 2 and 3. It was observed that pH, electrical conductivity (EC), total organic carbon (TOC), nitrogen content (N), phosphorus content (P), potassium (K), moisture content (MC), Total Petroleum Hydrocarbon (TPH) content and Total heterotrophic Bateria Counts(THB) changed immediately the crude oil was dispersed in the soils. These changes indicate the impact of crude oil on the soils, which affirmed the previous reports that the presence of crude oil in soil affected its physicochemical properties (Azubuike *et al*., 2016; Ere et al., 2020).

Parameters	Before Pollution	After Pollution	Control on day 84	Treated soil on day 84
pH	6.55	6.22	6.14	7.08
EC (µS/cm)	127.31	543.75	322.91	153.53
TOC (%)	3.86	6.38	4.12	2.44
N (%)	1.32	0.09	0.51	0.89
P (%)	1.98	0.002	0.26	0.46
K (%)	27.34	2.16	9.93	16.77
MC (%)	48.27	54.69	46.06	58.42
THB (cfu/g)	3690	384.37	883764	8745480
TPH(mg/kg)	217.26	35981.1	18746.1	1090.23

Table 2: Physicochemical characteristics of wet soil before and after treatment

Parameters	Before Pollution	After Pollution	Control on day 84	Treated soil on day 84
pH	6.74	6.35	6.26	7.28
EC (µS/cm)	115.96	482.53	282.35	149.55
TOC (%)	3.14	8.24	4.28	2.36
N (%)	0.91	0.07	0.47	0.85
P (%)	1.63	0.01	0.21	0.36
K (%)	24.71	1.48	6.17	13.19
MC (%)	6.95	13.62	13.39	13.66
THB (cfu/g)	1370	196.39	1943880	7642660
TPH(mg/kg)	88.54	32563.4	19090	1595.61

A. VARIATION OF SOIL pH

The trends in variation of pH in wet and dry soils over the period of the treatment is shown in in Figure 1.

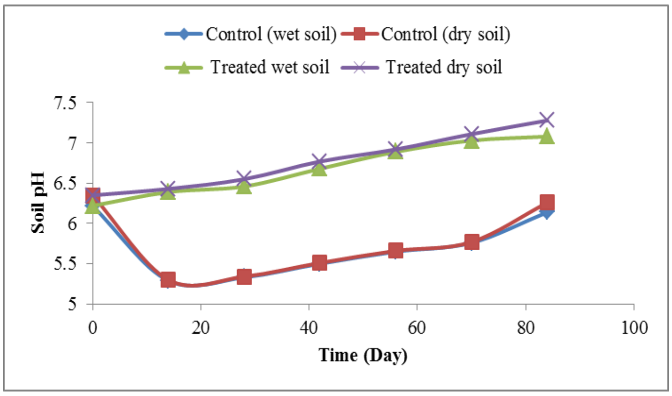

Figure 1: Variation of pH in wet and dry soils with time

Figure 1 shows the variation of pH in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. The pH of the soils decreased from its initial value after pollution, which was most evident in the control samples and least in sample with *Pseudomonas sp*. treatment (Table 2). Soil pH plays a crucial role in bioremediation of crude oil-polluted soil as it affects microbial activities and the availability of nutrients for microbial growth. Soil acidity can negatively impact the survival and activity of indigenous microorganisms that are essential for natural attenuation processes (Ayotamuno *et al*., 2006; Ere & Amagbo, 2019).

As shown in Table 2, pH of the soils reduced from 6.55 to 6.22 in wet soil and 6.74 to 6.35 in dry soil after pollution. However, the soil pH tends to return gradually to its original level as time increases. In the control samples, the pH further decreased to 5.29 and 5.30 in the wet and dry soil samples, respectively, at the 14th day before increasing gradually. The final pH recorded in the control samples on day 84 was 6.14 for wet soil and 6.26 for dry soil, which were still below the values recorded before the pollution. However, the pH values recorded in the treated wet and dry soils on day 84 were 7.08 and 7.28, respectively, which represents 13.83% and 14.65% increase in reference to values recorded after the pollution. The low pH in samples without treatment simply indicates that the intrinsic neutralization of the soil acidity was slow because of inhibited growth of native microbes (Pawar, 2015). Nevertheless, the pH of the soil samples inoculated with *Pseudomonas sp*. were enhanced, indicating the effectiveness of *Pseudomonas sp*. in neutralizing the soil acidity caused by the mineralization of crude oil.

B. VARIATION OF ELECTRICAL CONDUCTIVITY

The trend in variation of EC in wet and dry soils over the period of the treatment is shown in in Figure 2.

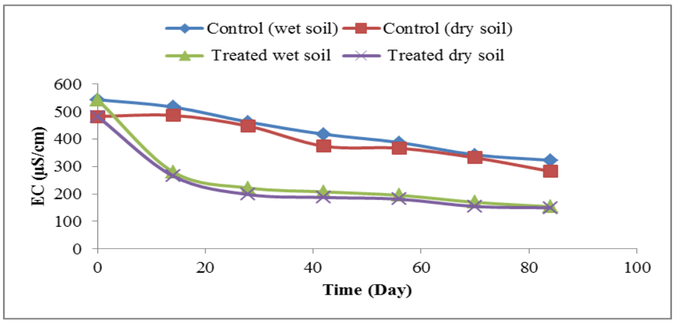

Figure 2: Variation of EC in wet and dry soils with time

Figure 2 shows the variation of electrical conductivity (EC) in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. There was a decrease in EC across the control and treated soil samples as the bioremediation time increases. Initially, the concentration of EC in the soils increased after crude oil pollution, but decreases as time progresses. The rate of decrease was more evident in treated soil samples, which is attributed to the utilization of nutrients by hydrocarbon degrading bacteria in the soil, thereby reducing the conductivity of the soil (Ere et al., 2020). Thus, EC of the soils before and after pollution were 127.31µS/cm and 543.75µS/cm for the wet soil and 115.96µS/cm and 482.53µS/cm for the dry soil (Table 2). After 84 days of treatment, the EC values decreased to 322.91µS/cm for wet soil control sample, 282.35µS/cm for dry soil control sample, while the wet and dry soil samples treated with *Pseudomonas sp*. reduced to 153.53µS/cm and 149.55µS/cm, respectively, representing 71.76% and 69.01% reduction in wet and dry soils, respectively. Similar studies have equally observed a decrease in EC of soil under treatment (Umeda et al., 2017 and Ere et al., 2020)

C. VARIATION OF TOTAL ORGANIC CARBON

The trends in variation of TOC in wet and dry soils over the period of the treatment is shown in in Figure 3.

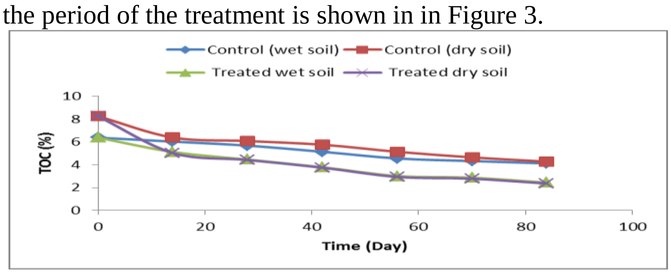

Figure 3: Variation of TOC in wet and dry soils with time

Figure 3 shows the variation of Total Organic Carbon (TOC) in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. As shown in the figure, TOC decreases with time across the treatment options including the control sample. The rate of decrease of TOC in the control sample was slower than samples with treatment. The speedy rate of decrease in TOC in samples with treatment was attributed to the utilisation of TOC for energy by micro-organism (Umeda *et al*., 2017). The value of TOC recorded in wet soil before and after pollution was 3.86% and 6.38%, but decreased to 4.12% in control sample and 2.44% in treated soil sample after 84 days. Similarly, TOC recorded in dry soil before and after pollution was 3.14% and 8.24%, but decreased to 4.28% in control sample and 2.36% in treated soil sample after 84 days. It was observed that the TOC levels in soils with treatment were below the values recorded before the pollution (Table 2). The reduction in TOC for the treated wet and dry soil samples are 61.76% and 71.36%, respectively. This indicates that TOC reduced more in dry soil compared to wet soil under treatment.

a. INFLUENCE OF TREATMENT ON THE SOIL TOTAL NITROGEN

The trends in variation of total nitrogen in wet and dry soils over the period of the treatment is shown in in Figure 4.

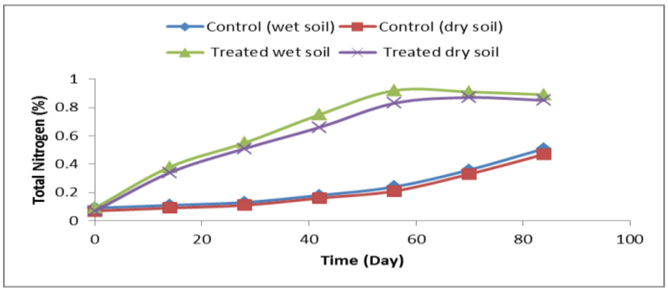

Figure 4: Variation of total nitrogen in wet and dry soils with time

Figure 4 shows the variation of total nitrogen in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. Obviously, total nitrogen increased in the soil samples, particularly, in treated soil samples. This will contribute to the rate of TPH degradation since nitrogen is an important nutrient for hydrocarbon degrading bacteria (Das & Chandran, 2011; Al-Hawash *et al*., 2018). The total nitrogen recorded before and after pollution was 1.32% and 0.09% for wet soil and 0.91% and 0.07% for dry soil, but under treatment, it increased to a maximum value of 0.92% in day 56 for wet soil and 0.87% in day 70 for dry soil. The total nitrogen also increases in the control soil samples, though, at slower rate. The maximum increase was recorded in day 84 with 0.51% for wet soil and 0.47% for dry soil.

After the optimum point, nitrogen content declined slightly for the rest of the days. This situation is expected because nitrogen is being utilised by hydrocarbon degrading bacteria (Al-Hawash *et al*., 2018; Ere *et al*., 2020). Besides being utilized by microorganism, the rate of nitrogen depletion can be aided by the presence of oxygen, which also leads to high degradation rate of TPH (Umeda *et al*., 2017). However, within timeframe of the analysis, the percentage of total nitrogen recorded across the samples is still below the initial value before the pollution. This may be due to the utilisation of the nitrogen by hydrocarbon degrading bacteria in soil.

b. INFLUENCE OF TREATMENT ON THE SOIL PHOSPHORUS CONTENT

The trends in variation of phosphorus in wet and dry soils over the period of the treatment is shown in in Figure 5.

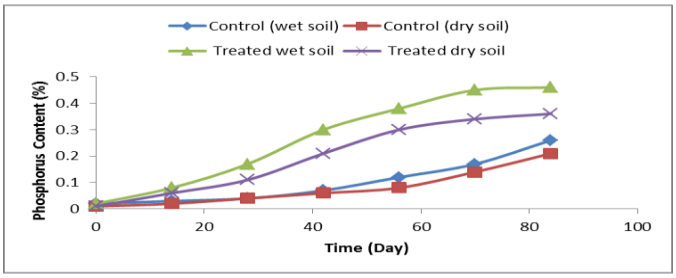

Figure 5: Variation of phosphorus in wet and dry soils with time

Figure 4.9 shows the time variation of phosphorus content in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. Phosphorus content increased with increase in time, but low values were recorded in the control samples. Phosphorus is another important nutrient required for microbial growth during bioremediation, and increase in phosphorus content also boosts microbial activities to favour the rate of TPH degradation (Nikolopoulou *et al*., 2013). The value of phosphorus recorded before and after pollution was 1.98% and 0.002% for wet soil and 1.63% and 0.01% for dry soil. However, under treatment, the levels increased gradually to a maximum value of 0.46% for wet soil and 0.36% for dry soil in day 84. In the control soil samples, the maximum value was also recorded in day 84 with 0.26% for wet soil and 0.21% for dry soil. This result is consistent with findings from other studies (Adaba, 2013; Umeda *et al*., 2017).

c. INFLUENCE OF TREATMENT ON THE SOIL POTASSIUM

Potassium result obtained over the period of the treatment is plotted in Figure 6.

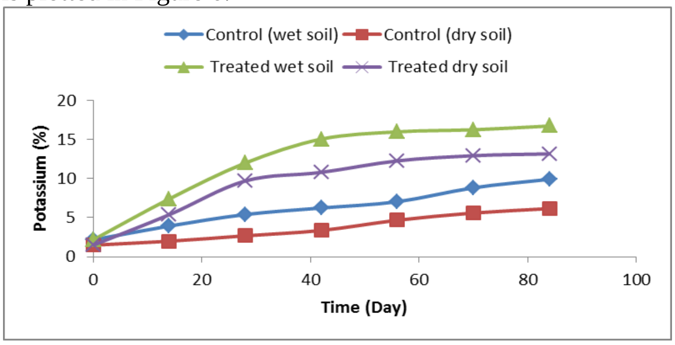

Figure 6: Variation of potassium in wet and dry soils with time

Figure 6 shows the time variation for potassium content in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. Like nitrogen and phosphorus, potassium is also used as nutrient for hydrocarbon degrading bacteria, but the concentration recorded was higher than nitrogen and phosphorus. Potassium content in the soil also increases with increase in time. The value of potassium recorded before and after pollution was 27.34% and 2.16% for wet soil and 24.71% and 1.48% for dry soil. During the treatment, the levels increased to a maximum value of 16.77% for wet soil and 13.19% for dry soil in day 84. Similarly in the control soil samples, the maximum value was also recorded in day 84 with 9.93% for wet soil and 6.17% for dry soil. The

ISSN: 2394-4404

increase in treated samples is expected because of the influence of *Pseudomonas sp*. (Sihag *et al*., 2011).

d. INFLUENCE OF TREATMENT ON MOISTURE CONTENT OF SOIL

The moisture content in the soils over the period of the treatment is plotted in Figure 7.

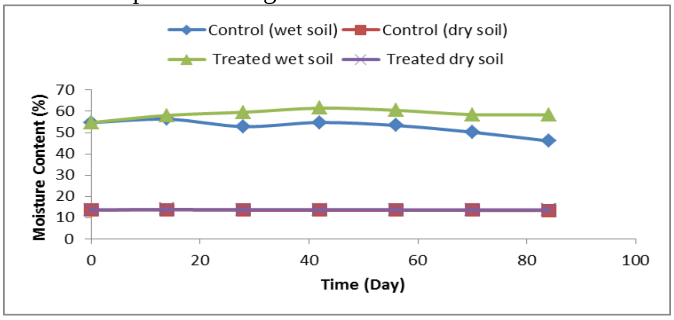

Figure 7: Variation of moisture content in wet and dry soils with time

Figure 7 shows the profiles of moisture content (MC) versus time for treated and untreated soil samples. Moisture content only marginally varied across the samples. The value of moisture content recorded before and after pollution was 48.27% and 54.69% for wet soil and 6.95% and 13.62% for dry soil. These results revealed that moisture content in the wet sample was far above the dry soil, and this is expected considering that the wet soil was collected in the swamp. At the end of the analysis, the moisture content recorded in the control soil sample was 46.06% for wet soil and 13.39% for dry soil, while with *Pseudomonas sp*. treated soils the moisture content was 58.42% for wet soil and 13.66% for dry soil (Table 2). The results are consistent with other studies using different treatments in crude oil polluted soil (Umeda *et al*., 2017; Ere *et al*., 2020). It is worthy of note that moisture is an important factor in bioremediation process as it limits the effectiveness of amendment in biodegradation of petroleum hydrocarbon contaminants (Hamoudi-Belarbi *et al*., 2018).

D. TOTAL HETEROTROPHIC BACTERIA COUNT

The variation of growth rate of Total Heterotrophic Bacteria (THB) in wet and dry soils over the period of the treatment is depicted in Figure 8.

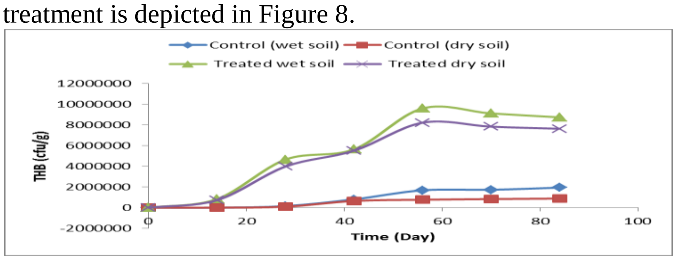

Figure 8: Variation of THB Count in wet and dry soils with time

Figure 8 shows the growth rate of Total Heterotrophic Bacteria (THB) in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. The Total Heterotrophic Bacteria (THB) count recorded in the soil before pollution decreased immediately the soil was contamination with crude oil, but started to increase as However, as the bioremediation time increases. There was remarkable increase in population of THB as time increases especially in the samples treated with *Pseudomonas sp*. Maximum population growth of THB was recorded in day 56 for the treated samples, as there a slight decline with further increase in time, while in the control sample, the maximum THB was recorded in day 84. As shown in Table 2, the THB recorded before and after pollution were 3.69 x 10^3cfu/g and 3.84 x 10^2cfu/g for wet soil and 1.37 x 10^3cfu/g and 1.96 x 10^3cfu/g for dry soil. The maximum THB recorded in the treated sample was 9.6092 x 10^6cfu/g for wet soil and 8.2284 x 10^6cfu/g (day 56), which reduced to 8.7455 x 10^6cfu/g and 7.6423 x 10^6cfu/g for wet and dry soil, respectively. For control samples, the maximum THB growth was recorded in day 84, with values recorded as 1.9439 x 10^6cfu/g for wet soil and 8.8376 x 10^5cfu/g for dry soil.

The THB recorded agreed with the trend reported in previous studies for soils amended by animal dropping and spent mushroom (Olu, 2017; Ere and Amagbo, 2019). The level of bacteria growth recorded in the treated samples is attributed to the *Pseudomonas sp*. However, the microbial growth rate is more enhanced in the wet soil. This was due to the presence and bioavailability of nitrogen and phosphorus in the soil which were sources of nutrient for microbial communities (Olu, 2017; Ere *et al*., 2020). The growth in bacteria favours the biodegradation of petroleum hydrocarbons (Ani *et al*., 2019).

E. IMPACT OF TREATMENT ON DEGRADATION OF TOTAL PETROLEUM HYDROCARBON

The percentage degradation of TPH in wet and dry soils over the period of the treatment is depicted in Figure 9.

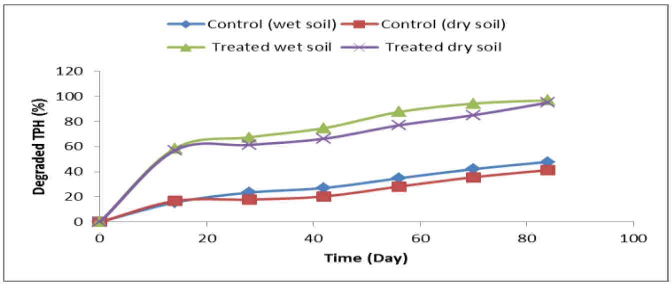

Figure 9: Percentage of TPH Degradation in wet and dry soils with time

Figure 9 shows the percentage of TPH degraded over time in the polluted soils under *Pseudomonas sp*. treatment in comparison with natural attenuation. The profiles indicated that increase in time simultaneously resulted in reduction of TPH concentration and consequence increase in percentage removal. As shown in Table 2, the TPH concentration recorded before and after pollution were 217.26mg/kg and 35981.09mg/kg for wet soil and 88.54mg/kg and 32563.39mg/kg for dry soil. However, during the bioremediation process, the specific concentration of TPH degraded considerably in the treated soil sample to 1089.15mg/kg for wet soil and 1595.61mg/kg for dry soil in day 84, representing 96.97% and 95.10%, respectively of TPH degraded in the treated soil sample. Also, the specific concentrations of TPH recorded in the control samples were 18746.1mg/kg for wet soil and 19090.03mg/kg for dry soil in day 84, representing 47.90% and 41.38%, respectively of TPH degraded in the control soil samples. There is remarkable removal of TPH from the soil with *Pseudomonas sp*. compared to the natural degradation, implying that the rate of TPH removal from the soil is slow under natural attenuation.

In a study using *Bacillus subtilis* and *Candida bombicola* as treatment agents reported 68.27% after 40 days (Yu *et al*., 2020), which affirmed the efficacy of microorganism in degrading TPH in polluted soil. The results were also consistent with several bioremediation studies amended with microorganisms or biostimulants (Akpe *et al*., 2015; Ogu and Odo, 2015; Hamoudi-Belarbi *et al*., 2018; Ere *et al*., 2020). Generally, the differences in the level of TPH removed from the treated soil samples as compared to the control samples shows the crude oil polluted soil inoculated by *Pseudomonas sp*. can restore the properties of the soil within a shorter time, would have been prolonged if left to degrade naturally.

IV. CONCLUSION

The present study demonstrated the bioremediation efficacy of indigenous *Pseudomonas sp*. for crude oil polluted wet and dry soils. The *Pseudomonas sp.* significantly improved the monitored parameters over the treatment period of 84 days compared to natural attenuation. The soils under treatment provided optimal conditions for microbial activity to thrive, which stimulated the prolific growth of the indigenous microorganism (THB), thereby increasing the biodegradation rate in wet and dry soils.

Remarkably, the addition of *Pseudomonas sp* lead high reduction of TPH within 84 days with over 95 % removal obtained across the treated soil samples, far exceeding the natural degradation process. Overall, *Pseudomonas sp*. proved to be a promising bioremediating agent, facilitating rapid and complete biodegradation of pollutants at optimized conditions. Therefore, bioremediation with *Pseudomonas sp*. can serve as an eco-friendly and low-cost remediation approach at crude oil polluted sites.

REFERENCES

[1] Adaba, C.S. (2013). Effects of Particle Size Distribution on Bioremediation of Crude Oil Polluted Sandy Soils, Nigerian Journal of Technology, 32(3), 435-439.

[2] Akpe, A.R., Ekundayo, A.O., Aigere, S.P. & 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.

[3] Al-Hawash, A.B., Dragh, M.A., Li, S., Alhujaily, A., Abbood, H.A., Zhang, X. & Ma, F. (2018). Principles of Microbial Degradation of Petroleum Hydrocarbons in the Environment, Egyptian Journal of Aquatic Research, 44, 71-76.

[4] Ani, E., Olofin, D.E., Okunlola, O.E. & Faniyi, A.F. (2019). Bioremediation of Hydrocarbon Contaminated Soil by Intercropping Luffa aegyptiaca with Vernonia amygdalina, Ameliorated with Growth Promoting Fungi, Singapore Journal of Scientific Research, 9, 33-44.

[5] ASTM D7066-04 (2015). Standard Practice for Total Petroleum Hydrocarbons by Stabilized Thermal Conductivity. ASTM International, West Conshohocken, PA.

[6] Ayotamuno, M.J., Kogbara, R.B. & Agunwamba, J.C. (2006).Bioremediation of a Petroleum Hydrocarbon Polluted Agricultural Soil at Various Level of Soil Tillage in Port Harcourt, Nigeria Journal of Technology, 25, 44-51.

[7] Azubuike, C.C., Chikere, C.B. & Okpokwasili, G.C. (2016). Bioremediation Techniques–Classification Based on Site of Application: Principles, Advantages, Limitations and Prospects, World Journal of Microbiology and Biotechnology, 32,180-197.

[8] Bertollo, F.B., Lopes, G.C. & Silva, E.L. (2018). Phenol Biodegradation by Pseudomonas putida in an Airlift Reactor: Assessment of Kinetic, Hydrodynamic, and Mass Transfer Parameters, Water, Air, & Soil Pollution, 228(10), 398-404.

[9] Borah, D. & Yadav, R.N.S. (2016). Bioremediation of Petroleum Based Contaminants with Biosurfactant Produced by a Newly Isolated Petroleum Oil Degrading Bacterial Strain Egyptian Journal of Petroleum, 26, 181-188.

[10] Das, N. & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview, Biotechnology Research International, 4(6), 810-822.

[11] Ebadi, A., Khoshkholgh-Sima, N.A., Olamaee, M., Hashemi, M. & Ghorbani-Nasrabadi, R. (2017). Effective Bioremediation of a Petroleum-Polluted Saline Soil by a Surfactant-Producing Pseudomonas Aeruginosa Consortium, Journal of Advanced Research, 8(6), 627-633.

[12] Ere, W. & Amagbo, L.G. (2019). Degradation Efficiency of Spent Mushroom in Petroleum Contaminated Soil, International Journal of Advanced Academic Research, 59(3), 17-23.

[13] Ere, W., Chie-Amadi, G.O., & Amagbo, L.G. (2020). Variations in Properties of Hydrocarbon Contaminated Soil under Bio-Wastes Treatments. International Journal of Advanced Academic Research | Sciences, Technology and Engineering, 6(5), 52-

[14] Galdames, A., Mendoza, A., Orueta, M., de Soto García, I.S., Sánchez, M., Virto, I. & Vilas, J.L. (2017). Development of New Remediation Technologies for Contaminated Soils Based on the Application of Zero-Valent Iron Nanoparticles and Bioremediation with Compost, Resource-Efficient Technologies, 3, 166 - 176.

[15] Hamoudi-Belarbi, L., Hamoudi, S., Belkacemi, K., Nouri, L., Bendifallah, L. & Khodja, M. (2018). Bioremediation of Polluted Soil Sites with Crude Oil Hydrocarbons Using Carrot Peel Waste, Environments, 5, 124-135.

[16] Nikolopoulou, M., Pasadakis, N., Norf, H. & Kalogerakis, N. (2013). Enhanced Ex Situ Bioremediation of Crude Oil Contaminated Beach Sand by Supplementation with Nutrients and Rhamnolipids, Marine Pollution Bulletin, 77, 37–44.

[17] Ogu, G.I. & Odo, B.B. (2015). Crude Oil Bioremediation Efficiency of Indigenous Soil Fungal Community Spiked with Cassava Peels in Niger Delta Region, Nigeria, The International Journal of Science & Technoledge, 3(12), 19-26.

[18] Olu, A.A. (2017). Effectiveness of Organic Fertilizer as a Biostimulating Agent for the Removal of Naphthalene in Soil, Applied Journal of Environmental Engineering Science, 4(1), 1-12.

[19] Pawar, R.M. (2015). The Effect of Soil pH on Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHS), Journal of Bioremediation & Biodegradation, 6(291), 23-44.

[20] Sihag, S., Pathak, H. & Jaroli, D.P. (2011). Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons, Internatiional Journal of Pure & Applied Bioscience, 2(3), 185-202.

[21] Umeda, U., Puyate, Y.T., Dagde, K.K. & Ehirim, E.O. (2017). Effect of Oxygen Diffusion on Physicochemical Properties of Petroleum Contaminated Sandy Soil, International Journal of Agriculture and Earth Science, 3(7), 1-9.

[22] Umeojiakor, C.T, Ojiabo, K.T., Umeojiakor, A.O., Anyikwa, S.O. & Nwanwe, C.C. (2019). Effectiveness of Biostimulants Amendment with Indigenous Microbes on Bioremediation of Crude Oil Contaminated Soil in Niger Delta Region of Nigeria, International Journal of Engineering Research & Technology, 8(11), 751-755.

[23] Varjani, S.J. & Upasani, V.N. (2017). A New Look on Factors Affecting the Microbial Degradation of Petroleum Hydrocarbon Pollutants, Biodeterioration & Biodegradation Journal, 120, 71-83.

[24] Wang, M., Zhang, B., Li, G., Wu T. & Sun, D. (2019). Efficient Remediation of Crude Oil-Contaminated Soil using a Solvent/Surfactant System, Royal Society of Chemistry Advances, 9, 2402–2411.

[25] Yu, Y., Zhang, Y., Zhao, N., Guo, J., Xu, W., Ma, M. & Li, X. (2020). Remediation of Crude Oil-Polluted Soil by the Bacterial Rhizosphere Community of Suaeda Salsa Revealed by 16S rRNA Genes, International Journal of Environmental Research and Public Health, 17, 1471-1488Ere et al., 2020).