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Comparison of TPH Removal from Crude Oil Polluted Soil under Pseudomonas *sp.* Treatment

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ABSTRACT

This study investigated and compared the biodegradation of total petroleum hydrocarbons (TPH) in crude oil contaminated wet and dry soils treated with Pseudomonas sp. The physicochemical properties of the soils were analyzed before and during treatment. TPH removal was assessed in soils treated with 0 – 50 ml of Pseudomonas sp. over 84 days. Results showed TPH degradation up to 96.97% and 95.10% in wet and dry soils treated with 50 ml Pseudomonas sp., respectively. TPH reduction was slightly higher in wet soil compared to dry soil across all treatments. The mean TPH degradation difference between wet and dry soils increased from 3.000% at 20 ml to 7.451% at 40 ml treatment. Moisture content, nutrients and microbial population influenced the higher biodegradation in wet soil. Overall, treatment of crude oil polluted soils with Pseudomonas sp. significantly enhanced the remediation of TPH in both wet and dry polluted soils. Therefore, the application of Pseudomonas sp. in crude oil polluted soil can restore the natural properties of soil at a reduce time compared to natural attenuation.

***KEYWORDS**: Bioremediation, TPH removal, Pseudomonas sp., Wet soil, Dry soil*

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I. INTRODUCTION

Petroleum hydrocarbon contamination of soil from exploration, transportation and storage activities is a major environmental concern (Varjani and Upasani, 2017). Total petroleum hydrocarbons (TPH) constitute the major fraction of crude oil that persists in the environment (Agarry and Ogunleye, 2012). Developing effective remediation techniques for cleaning up TPH contaminated sites is crucial (Ere and Amagbo, 2019).

Bioremediation utilizing microorganisms provides an eco-friendly approach for degrading TPH into less toxic compounds (Umeojiakor *et al*., 2019). Biostimulation using microbial amendments has shown high potential for enhancing biodegradation of petroleum hydrocarbons (Varjani *et al*., 2017). However, TPH degradation efficiency can vary based on soil physicochemical characteristics like texture, moisture content and nutrient levels (Galdames *et al*., 2017; Ere *et al*., 2020).

The Niger Delta region of Nigeria has over the years attracted internal attention in exploiting the vast crude oil reverse in the region. For decades, many international oil and gas firms have consistently explored and produced crude oil across the states in Niger Delta, including Bayelsa State. The terrain of Bayelsa State can best be described as wet because of the swampy characteristics of most Towns and Village settlements. Because of the consistent crude oil and gas activities, these communities are often polluted by crude oil. In the event of crude oil pollution, the environment, most times, are not scientifically attended to. This situation leaves the agricultural soil with many challenges including low fertility due to loss its natural properties. Hence, this study evaluated and compared the biodegradation of TPH in crude oil contaminated wet and dry soils treated with Pseudomonas sp. The changes in soil physicochemical properties were analyzed during the remediation process. The findings provide insights into the influence of soil conditions on TPH removal by microbial amendment.

II. MATERIALS AND METHODS

2.1 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 five samples and added into different containers (biocells). The biocells received 0 (control sample), 20, 30, 40 and 50ml of *Pseudomonas sp*. treatment. The sample design preparation is shown in Table 1.

Table 3.1: Experimental Design

Sample	Description
Control	Addition of 5kg of soil, 340ml of crude oil.
Reactor with 20ml	Addition of 5kg of soil, 340ml of crude oil, 20ml of *Pseudomonas sp* .
Reactor with 30ml	Addition of 5kg of soil, 340ml of crude oil, 30ml of *Pseudomonas sp*.
Reactor with 40ml	Addition of 5kg of soil, 340ml of crude oil, 40ml of *Pseudomonas sp*.
Reactor with 50ml	Addition of 5kg of soil, 340ml of crude oil, 50ml of *Pseudomonas sp*

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 for 84 days.

2.2 Soil Physicochemical and Bacteria Analysis

The soil particle size distribution was analyzed to classify the wet and dry soils. Physicochemical parameters like 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).

2.3 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:

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

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

3.1 Soil Characterization

Table 2 shows the soil classification, while Table 3 shows the physicochemical properties of the soils before and immediately after pollution.

Table 2: Soil Classification

Soil Sample	Bulk Weight (g)	Sand (g)	Silt (g)	Clay (g)
Wet Soil	500	112 (22.4%)	226 (45.2%)	162 (32.4%)
Dry Soil	500	175 (35%)	200 (40%)	125 (25%)

Table 3: Results analysis of soils before and after contamination

Parameters	Wet Soil		Dry Soil	
	Before Pollution	**After Pollution**	**Before Pollution**	**After Pollution**
pH	6.55	6.22	6.74	6.35
EC (µS/cm)	127.31	543.75	115.96	482.53
TOC (%)	3.86	6.38	3.14	8.24
N (%)	1.32	0.09	0.91	0.07
P (%)	1.98	0.002	1.63	0.01
K (%)	27.34	2.16	24.71	1.48
MC (%)	48.27	54.69	6.95	13.62
TPH(mg/kg)	217.26	35981.09	88.54	32563.39
THB (cfu/g)	3.69×10^3	3.8437×10^2	1.37×10^3	1.9639×10^2

Based on the particle size distribution in Table 2, the wet and dry soils were classified as clay loam and silt loam respectively. Table 3 shows that the pollution of the by crude oil substrate markedly increased TPH, TOC, EC and MC, while N, P, K and THB reduced in both soils. The wet soil had higher nutrients and moisture than the dry soil. Ere et al. (2020) showed that the pollution of soil with crude oil immediately altered the properties of the soil. This alteration affects soil fertility and also disrupts the existence of macro and microorganisms, which some worst case scenario, it may even their dead.

3.2 TPH Degradation

Table 4 shows percentage of TPH degradation recorded at various times during the treatment process including the control samples for both wet and dry soils, while the profiles are shown in Figures 1 to 5.

Table 4: Comparison of TPH Degradation in Wet and Dry Soil

Time (Days)	TPH Degradation (%)									
	Control		**20ml**		**30ml**		**40ml**		**50ml**	
	Wet Soil	Dry Soil	Wet Soil	Dry Soil	Wet Soil	Dry Soil	Wet Soil	Dry Soil	Wet Soil	Dry Soil
0	0	0	0	0	0	0	0	0	0	0
14	15.54	16.71	42.49	39.41	45.57	45.18	56.08	50.53	58.40	56.91
28	23.44	17.86	49.65	46.88	54.54	50.81	62.14	57.11	67.49	61.37
42	27.09	20.37	55.88	53.14	62.21	57.32	71.99	62.35	74.75	66.30
56	34.79	28.24	66.58	62.67	71.93	66.17	84.13	73.21	87.60	76.97
70	42.12	35.65	75.74	73.04	80.52	76.34	92.44	81.79	94.30	85.06
84	47.9	41.38	89.44	86.63	92.96	89.48	94.76	91.85	96.97	95.10

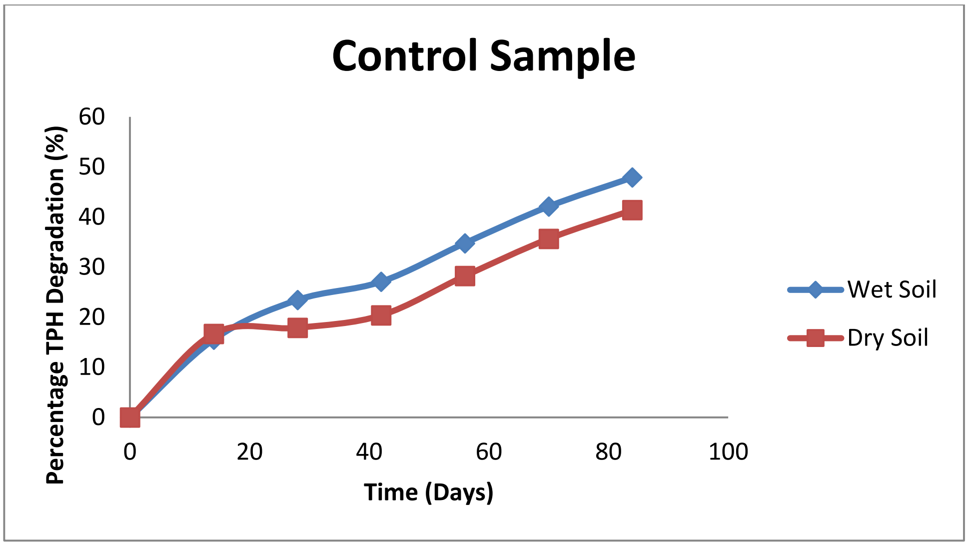

Figure 1: Comparison of TPH Degradation in Wet and Dry Soil for Control Sample

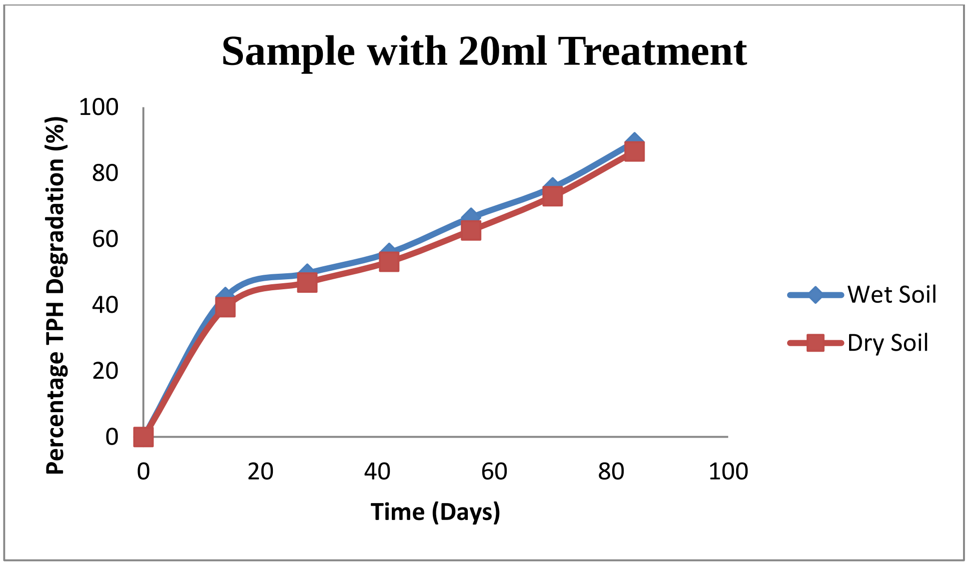

Figure 2: Comparison of TPH Degradation in Wet and Dry Soil at 20ml Treatment

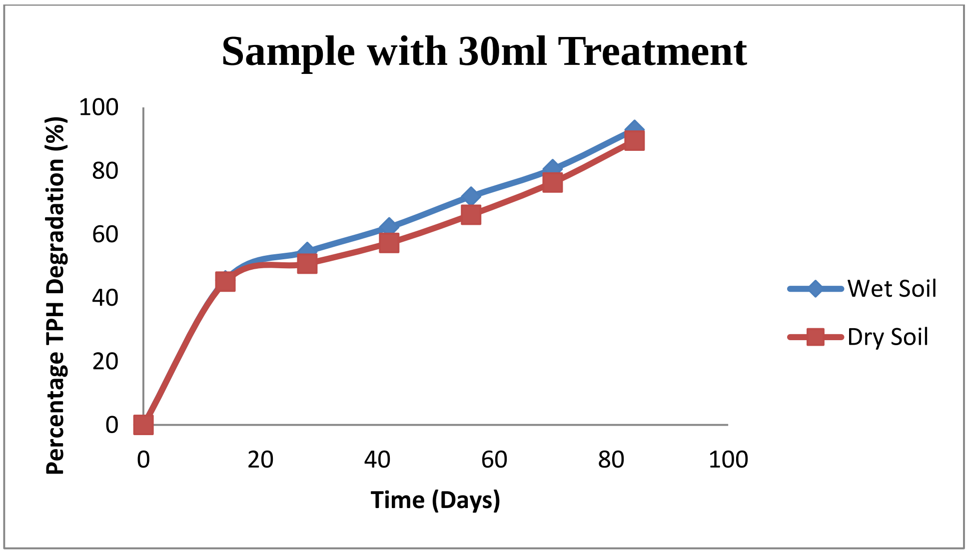

Figure 3: Comparison of TPH Degradation in Wet and Dry Soil at 30ml Treatment

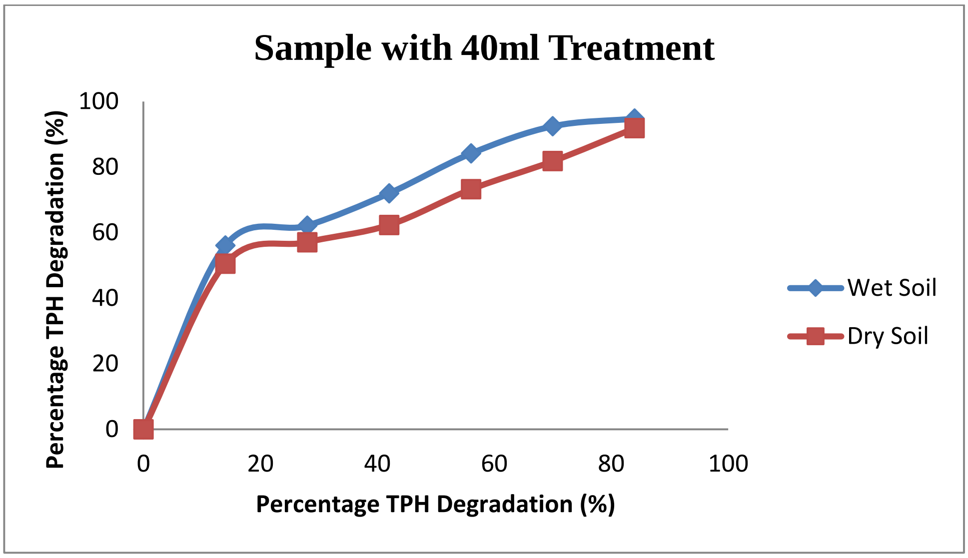

Figure 4: Comparison of TPH Degradation in Wet and Dry Soil at 40ml Treatment

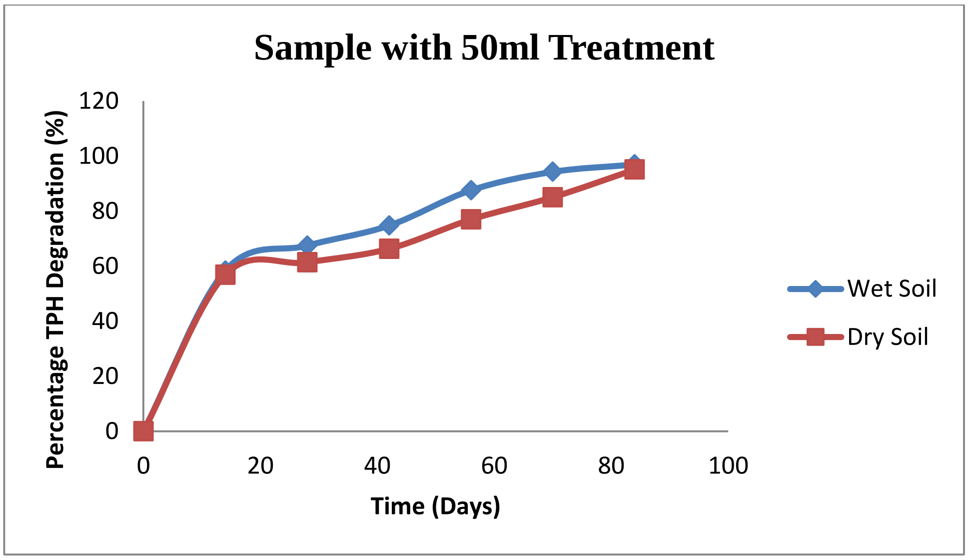

Figure 5: Comparison of TPH Degradation in Wet and Dry Soil at 50ml Treatment

Figures 1 to 5 show the comparison of TPH degradation in wet and dry soils for natural attenuation and samples treated with 20 ml, 30 ml, 40ml and 50 ml of *Pseudomonas sp*. over 84 days of bioremediation. Across the various treatments, including the control sample, the percentage of TPH removed from the wet soil was slightly higher than the percentages removed from the dry soil. Over the period of the analysis from 14 to 84 days, the percentage of TPH removed in the untreated soil samples increased from 15.54 to 47.90% for wet soil and 16.71 to 41.38% for dry soil (Table 4).

For soil samples treated with 20ml of *Pseudomonas sp.*, the percentage of TPH removed increased from 42.49 to 89.44% for wet soil and 39.41 to 86.63% for dry soil, while for soil samples treated with 30ml of *Pseudomonas sp.*, TPH removed increased from 45.57 to 92.96% for wet soil and from 45.18 to 89.48% for dry soil. Also, the TPH removed from the soils amended with 40ml of *Pseudomonas sp*. increased from 56.08 to 94.76% for wet soil and from 50.53 to 91.85% for dry soil, while in the soils amended with 50ml of *Pseudomonas sp*., TPH removed increased from 67.49 to 96.97% for wet soil and from 61.37 to 95.10% for dry soil.

The results indicate that the level of TPH degradation in wet soil across the treatment options is slightly greater than that of dry soil. This may be attributed to higher moisture content, nutrient levels and microbial population which promoted microbial activity (Umeda et al., 2017; Ere *et al*., 2020). Several studies have also observed higher removal of TPH from soil samples at higher nutrients (Bandura *et al*., 2017; Mohammadi-Sichani *et al*., 2017). Overall, the degree of TPH degradation shows that *Pseudomonas sp*. is an effective bioremediation treatment for crude oil polluted soil.

IV. CONCLUSION

The nature of soil can affect the performance of treatment agent in removing total petroleum hydrocarbon. The *Pseudomonas sp*. applied for the treatment of crude oil polluted soils resulted in significantly removal of TPH, but it was slightly more effective in wet soil compared to dry soil which could be attributed to its higher moisture content, nutrients and microbial counts. Despite the variation, *Pseudomonas sp*. showed high potential for remediating TPH pollution in both wet and dry soils, and could be used between 30 and 50ml to achieve the bioremediation in shorter duration.

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