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Brackish Water as Biostimulant in the Degradation of Total Petroleum Hydrocarbon and Polycyclic Aromatic Hydrocarbon in Crude Oil Polluted Soil

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***Abstract**: Crude oil pollution has been connected to a rise in the degradation of environmental soils, depositing many contaminants like hydrocarbons and heavy metals and also making the environment unfavorable for living things. The dream of creating such items for the unsolvable environmental pollution circumstances of today becomes a reality with the application of locally sourced environmental remediation stimulants. By doing this, additional quantity can then be used to repair polluted soil. Despite having a high nutritional content, brackish water has proven difficult to use as biostimulants to improve soil. This study assessed the brackish water's capacity for bioremediation of soil contaminated with crude oil. The experiment was conducted at the teaching and research farm of the Rivers Institute of Agricultural Research at Rivers State University in Port-Harcourt. In five (5) cells (T1, T2, T3, T4, T5) with three replications each, soil samples containing crude oil were used. Particle size distribution (PSD), nitrogen, phosphorus (P), potassium (K), total petroleum hydrocarbon (TPH), total polycyclic aromatic hydrocarbon (TPAH), and total heterotrophic bacteria (THB) were among the physiochemical characteristics of treated soil and brackish water that were examined in the lab both before and after treatment. The findings demonstrated that the soil is a loamy sand soil and that brackish water has high NPK levels, which demonstrate that it is a biostimulant. At the conclusion of the remediation period of 8 weeks, TPH and PAH similarly had significant decreases in all treatment choices, with their percentage reductions exceeding 94% for the same duration. Additionally, the Analysis of Variance (ANOVA) results at 95% confidence levels revealed a significant difference. Additionally, during eight (8) weeks of repair, there was an increase in the number of THB, which then decreased as the stimulants were removed after reaching their peak. Therefore, it is advised that brackish water be utilized as an excellent biostimulant to encourage the breakdown of TPH and PAH in soil that has been polluted with crude oil.*

Keywords: bioremediation, biostimulant, brackish water, crude oil contaminated soil, total heterotrophic bacteria, total petroleum hydrocarbon, total polycyclic aromatic hydrocarbon

1. Introduction

When crude oil spills on land, which is a complex mixture of hundreds of hydrocarbons and non-hydrocarbon chemicals, it alters the physicochemical characteristics of the soil, including its pH, structure, temperature, and nutrient status. It is mainly made up of various hydrocarbon complex mixes [1]. Within the same environment, the chemical compositions might have a variety of impacts on various macro- and microorganisms. Recent years have seen a number of noteworthy studies on the remediation of soil contaminated by petroleum hydrocarbons carried out by experts all around the world. Bulk density, total porosity, pH, available phosphorus, total petroleum hydrocarbon (THC), organic matter, percent organic carbon, total nitrogen, exchangeable Ca, Na, and P, mg, ECEC, TEA, and base saturation were all found to be impacted by the amount of crude oil simulated into soil [2]. They also noted that, with the exception of porosity, all of the examined characteristics—including bulk density, pH, accessible P, THC, organic matter, percent organic carbon, total nitrogen, exchangeable cation (Ca, Na, P, Mg), ECEC, TEA, and base saturation increased as crude oil volume increased. Additionally, crude oil contamination might hinder crops' natural growth by lowering their fertility, germination rate, and resistance to pests and diseases [3]. Ekemube *et al*. [2] also discovered that crude oil changes the physicochemical properties of the soil; hence, increase in crude oil present in the soil, the more these properties change, leading to an imbalance in the nature of the soil. Additionally, their research indicated that remediation should be used to restore the soil's natural composition.

The typical techniques, according to Han [4], are plant remediation, chemistry, microbiology, and physical remediation. Traditional physical and chemical methods, such as soil removal and replacement, elution method, heat treatment and thermal resolution, extraction-separation, and chemical oxidation methods, are used to change the physical properties of the soil and effectively control and regulate contaminants [1]. These techniques are typically more thorough and reliable, but in order to handle the entire process, it is necessary to construct some fixed processing facilities, use chemical agents, continuously regulate temperature and pressure, and provide a regular power supply throughout the entire operation [4–6]. It is not frequently employed in the real application because of its high cost and secondary contamination, which makes it unsuitable for large-area remediation [1]. In contrast, bioremediation technology has been demonstrated to be more suitable for the remediation of petroleum-contaminated soil due to its low cost, straightforward in-situ and ex-situ treatments, environmental friendliness, lack of secondary pollution, and high efficiency in removing the majority of pollutants [7–9]. In order to remediate soil that had been contaminated by crude oil, Ekemube et al. [10] used spent mushroom substrate (SMS) and NPK fertilizer as biostimulants. They discovered that the removal of total petroleum hydrocarbon (TPH) concentration across treatment cells was a result of varying amounts of SMS, NPK fertilizer, and changes in microbial activities.

The use of brackish water as locally available source of nutrient for the thriver of microorganisms in a crude oil polluted soil was applied in this study Brackish water is also known as saline water, salinity refers to the concentration of dissolved salt in pure water and its salty concentration is caused by the evaporation of sea and ocean water through the hydrological cycle. This is influenced by the rate of evaporation, supply of fresh water through rivers, supply of melt water, wind, ocean currents and rainfall. The most common compounds found in saline water are sodium, chlorine, magnesium, calcium and potassium. Saline water also contains dissolved atmospheric gases. The carbonates in the water are consumed by animals whereas the silicates are consumed by plants hence there is a general concentration of sulphates and chlorides in the water. These makes it to have a high nutrient value that cause its attraction for bioremediation of a crude oil polluted soil. It has been abundant in the study area, but has not been used as biostimulant for remediation purposes. Despite its potential, brackish water's value in cleaning up polluted soils has not been well-documented in the literature. Consequently, this investigation is necessary. Therefore, the aim of this study is to assess the potential impact of brackish water in the degradation of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH) in soil polluted with crude oil.

2. MATERIALS AND METHODS

2.1 Study Area Description

The experiment was conducted at Rivers State University in Port Harcourt, Nigeria's Rivers Institute of Agricultural Research Teaching and Research Farm. The capital of Rivers State and primarily the hub of crude oil exploration for both the Niger Delta and all of Nigeria is Port Harcourt. The Port Harcourt metropolis' ambient atmosphere has a mean monthly relative humidity of 85%, a daily minimum temperature of roughly 230 degrees Celsius, and a mean daily maximum temperature of 32 degrees Celsius.

2.2 Sample Collection

Loamy sand soil from the teaching and research farm of Rivers State University in Port Harcourt, Nigeria, was gathered for this study. A local oil and gas exploration and exploitation firm allowed for the collection of crude oil. In Port Harcourt, brackish water was obtained from a river that had never previously been contaminated by crude oil.

2.3 Experimental Design

The experimental design that adopted is completely randomized design (CRD) for single factor experiment. The design consisted of 5 experimental reactors including the control T1 with three replications. Here, randomization will be achieved using the draw lots approach as described in several [11 -13]. Each reactor contained 10kg of soil with a depth of 15cm. The primary functions of the reactors are to control the surface area of the soil, the nutrients concentration, moisture content, temperature and oxygen availability. They also serve to prevent excessive run-off of the contaminant, which is a common trend in the locality during the period the study will be conducted. Cell T1 receives 100ml of water two times per week; Cell T2 receives 100ml of brackish water; Cell T3 receives 200 ml of brackish water; Cell T4 receives 300 ml of brackish water; and Cell T5 400 ml of brackish water.

2.4 Sample Preparation

50,000mg of Bonny light crude oil was poured on each treatment cells (including the control cell, T1) through a perforated can at the rate of 50,000mg of crude oil per 10kg of soil and the surface of the soil in each cell will be completely covered with a thin layer of oil. The objective is to stimulate condition of a major spill. The cells were left undisturbed for 72 hours. After the 72 hours, treatment options were applied. Brackish water was harvested from uncontaminated river without any history of contamination, and to be used for cell T2, T3, T4, and T5, respectively. The brackish water was applied after 72 hours of bulking, once for the 8 weeks of remediation period. Different random spots were augured using hand dug soil auger capable of obtaining uniform cores of equal volume to desired depth, bulked together (composite soil samples) and put in well labeled sample bottles. This was carried out at the interval of two (2) weeks each for eight (8) weeks. The physiochemical properties analysed for in the laboratory were total petroleum hydrocarbon (TPH), total polycyclic aromatic hydrocarbon (TPAH), and total heterotrophic bacteria (THB) in crude oil contaminated soils, respectively.

2.5 Laboratory Analysis

The following soil physicochemical parameters: particle size distribution, total petroleum hydrocarbon (TPH), and total heterotrophic bacteria (THB) was analyzed using standard method.

2.5.1 Particle Size Distribution (PSD)

The hydrometer method was used to determine the soil textural class in the laboratory. It was also determined using soil textural triangle according to United States Department of Agriculture (USDA), soil textural classification. Finally, the textural class was determined using soil triangle according to United State Department Agriculture (USDA) soil textural classification scheme using TAL for Windrows.

2.5.2 Nitrogen, Phosphorus and Potassium

Nitrogen was determine using the modified Kjeldahl method. Ascorbic acid method was used to estimate available phosphorus. While potassium content in the brackish water samples were analysed by flame atomic absorption spectrometry using a UNICAM-969 atomic spectrophotometer by measuring the light absorbance of the sample at the wavelength range of 357.9 – 228.8μm according to APHA method 3111C.

2.5.3 Petroleum Hydrocarbon (TPH)

TPH of the samples were analysed in line with USEPA 8015 method using Gen Tech master G equipped with a split / split less injector, J and W 30-meter DB-5column and an FID detector.

2.5.4 Total Polycyclic Aromatic Hydrocarbon ((TPAH)

PAH analysis was carried out in line with EPA 8270 method on an Agilent 6890 GC /MSED 5973 equipped with a split/splitless injector, J and W 30-meter DB-5 column and mass selective detector

2.5.5 Bacteria Count/Microbial Analysis

THB cultivation and enumeration was carried out using the method by Harrigan and McCane (1990), in addition the isolation, characterization, identification of bacteria in the soil was done with reference to Cowan [14]; Buchanam and Gibbons [15].

2.6 Statistics Analysis

The statistical method that was employed to analyze the data is analysis of variance (ANOVA). ANOVA is based on the F-test and it help to obtained an appropriate error term with single probability risk determined if the means considered are totally different and if the difference are beyond what is attributed to chance or experimental error sand difference will be considered as significant at $p \leq 0.05$.

3 RESULTS AND DISCUSSIONS

3.1 Physicochemical Composition of Uncontaminated Soil, Brackish Water

Table 1 displays the soil's physicochemical composition as well as its brackish and THB content. The physicochemical compositions were examined and utilized as indicators to gauge the degree of contamination in both the uncontaminated (original state) and contaminated (after crude oil) states. Unpolluted soil and brackish water are two possibilities. The physicochemical parameter followed was found to be more concentrated in soil than brackish water. It was therefore adopted for usage as a remediating agent.

Table 1: Physicochemical Composition of the Initial Soil Condition and Brackish Water

Parameters	Treatment Options	
	Unpolluted Soil	Brackish Water
N (mg/Kg)	12.65	8.46
P (mg/Kg)	1.89	1.02
K (mg/Kg)	6.768	8.24
TPH (mg/Kg)	84.23	N/A
PAH (mg/Kg)	<0.01	N/A
THB $X10^5$(cfu/mg)	2.3	2.7

3.1.1 Soil Textural Classification

For particle size distribution (PSD) analysis, soil samples from the soils under study that had not yet been contaminated by crude oil were taken using a soil corer. The analysis of the uncontaminated soil revealed that sand, silt, and clay were present in the soil in varying amounts (Table 1). The United States Department of Agriculture (USDA) textural classification of soil using TAL for Windows, a program used by the USDA to assess soil type, indicated from the results that the soil textures were loam.

Table 2: Particle Size Analysis Results

Sand %	Silt %	Clay %	Textural Class
12.60	6.80	80.60	Sandy loam

3.1.2 Fertilizing Value of Brackish Water

The initially identified NPK properties served as indices for the assessment of the brackish water's fertilizing capacity (i.e., remediation potential). Table 2 compares the brackish water's NPK values to the 3: 2.5: 0.5 (N: P: K) recommended fertilizer value for materials, with brackish water falling within acceptable ranges. This supports the idea that brackish water is a strong candidate for clean-up operations, which supports the utilization of the items.

Table 3: NPK Values of Brackish Water

Properties	Brackish Water
N (mg/kg)	8.46
P (mg/kg)	1.02
K (mg/kg)	8.24

3.2 Effect of Brackish Water Juice on TPH

Table 4 illustrates how brackish water affects the amount of TPH in soil that has been contaminated by crude oil. The graphical change of TPH concentration in brackish water-remediated soil contaminated with crude oil over time for several research cells is also shown in Fig. 1. When brackish water was added and the number of weeks the study was conducted, the TPH concentrations in the various cells (T1, T2, T3, T4, and T5) dropped, according to an analysis of Fig. 1. Different application rates of brackish water that wasn't diluted with water were used to alter the cells (T2, T3, T4, and T5). According to the order of reduction, the TPH concentrations reduced in the different cells (T1, T2, T3, T4, and T5) ranged from 16,382.12 to 8,858.53, 16,38.10 to 819.97, 16,38.15 to 606.39, 16,38.11 to 397.22 and 16,38.19 to 197.89 mg/kg for 0, 2, 4, 6, and 4 weeks, respectively. Table 5 displays the % drop in TPH brought on by the presence of brackish water as the number of weeks increases. According to Table 5 and Figure 2, the TPH percentage decreases for weeks 0, 2, 4, 6, and 8 varied from 0.00 to 45.93, 0.00 to 95.00, 0.00 to 96.30, 0.00 to 97.58, and 0.00 to 98.79%. This is brought on by the brackish water's application to the heavily nutrient-rich (NPK) soil that has been contaminated by crude oil. With a TPH degradation potential efficiency of 94.73, 96.10, 97.45, and 98.73% over the course of eight (8) weeks, brackish water was used in this study to bioremediate crude oil-contaminated soil. With a high degrading efficiency of 95% and above, this experiment was conducted utilizing four (4) distinct application rates (100, 200, 300, and 400 ml) of brackish water. Additionally, the TPH data showed that 400 ml of brackish water reduced the soil's TPH to 98.73%. This demonstrated that the treatment T5's application of a greater amount of brackish water resulted in a more effective degradation of TPH. According to the ANOVA results regarding the impact of brackish water on TPH concentration, there were significant differences between the treatment means at 5% levels of significance. This shows that there is a 95% chance that the variation in the volume of brackish water applied was what caused the variance in treatment means.

Table 4: Mean Results of TPH (mg/kg) Concentrations in Crude Oil Polluted Soil Remediated with Brackish Water

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	16382.12	16384.10	16384.15	16384.21	16384.19
2	12489.27	5838.22	4878.23	3841.31	2026.19
4	10892.85	3279.89	2225.14	1163.29	978.80
6	9852.63	1639.95	1484.49	1170.65	824.24
8	8858.53	819.97	606.39	397.22	197.89

Table 5: Mean Results of TPH Concentrations Reduction (%) in Brackish Water Remediated Soil

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	0.00	0.00	0.00	0.00	0.00
2	23.76	64.37	70.23	76.55	87.63
4	33.51	79.98	86.42	92.90	94.03
6	39.86	89.99	90.94	92.86	94.97
8	45.93	95.00	96.30	97.58	98.79

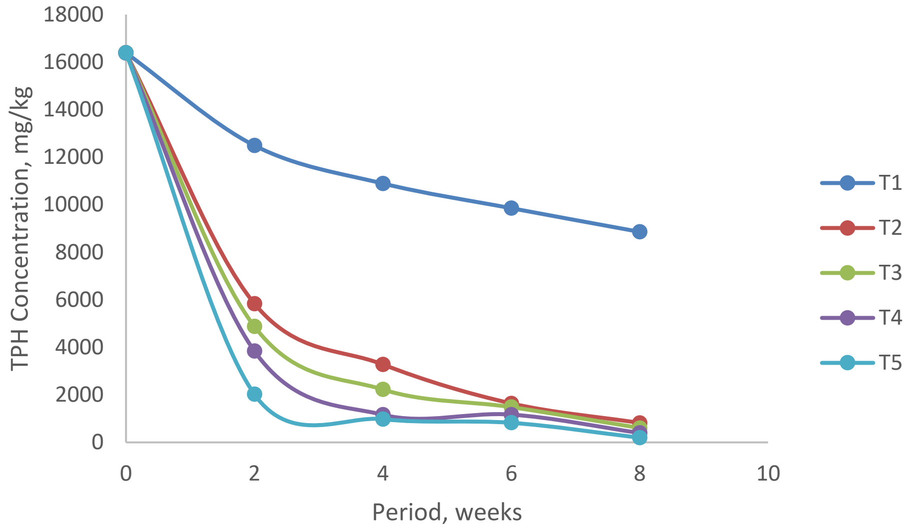

Fig. 1: Potential of Brackish Water on TPH Degradation in Crude Polluted Soil

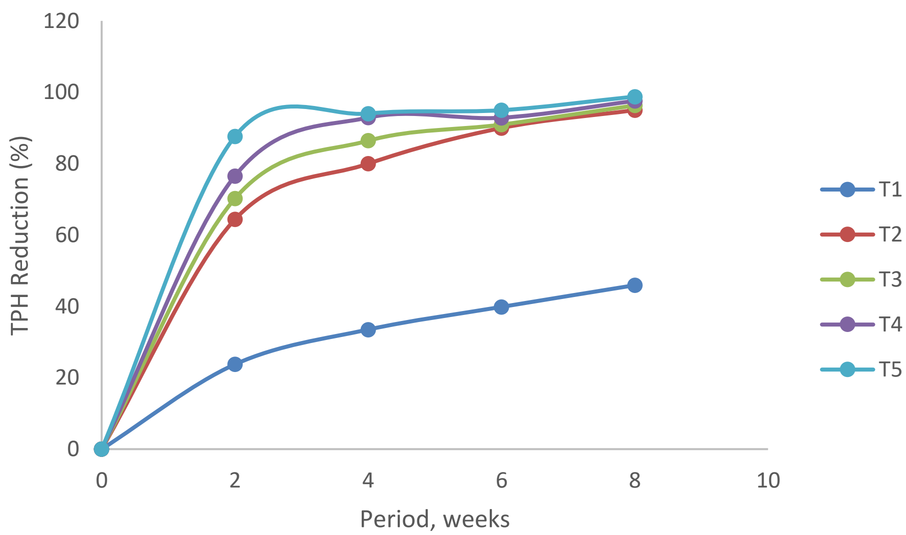

Fig. 2: TPH Reduction on Influence of Brackish Water in Crude Polluted Soil

3.3 Effect of Brackish Water on TPAH

It is demonstrated how brackish water affects the amount of TPAH in soil that has been contaminated by crude oil. The effect of brackish water on the TPAH levels in the crude oil-contaminated soil is shown in Table 5. Fig. 3 also displays the graphical evolution of the TPAH concentration in brackish water-remediated, crude oil-contaminated soil over time for the several cells used in this experiment. As the volume of brackish water and the length of weeks the study was done increased, Fig. 3 shows that the TPAH concentrations in the different cells (T1, T2, T3, T4, and T5) decreased. To the cells (T2, T3, T4, and T5), varied quantities of brackish water were supplied without dilution. The TPAH concentrations decreased in the various cells (T1, T2, T3, T4, and T5) in the following orders: 32.23.12 to 16.68, 32.20 to 1.82, 32.23 to 1.23, 32.18 to 0.98, and 32.22 to 0.88 mg/kg for 0 weeks, 2 weeks, 4 weeks, and 6 weeks, respectively. As the number of weeks rises, Table 7 shows the percentage drop in TPAH brought on by the presence of brackish water. In treatment cell T1, T2, T3, T4, and T5, the percentage decreases in TPAH for weeks 0, 2, 4, 6, and 8 ranged from 0.00 to 48.25, 0.00 to 94.34, 0.00 to 96.20, 0.00 to 96.96, and 0.00 to 97.26%, respectively. This is brought on by the presence of brackish water, which was applied to the soil contaminated with crude oil and contains a high amount of nutrients (NPK). In this study, brackish water, a locally accessible substance with TPAH degradation potential efficiencies of 94.04, 96.00, 96.80, and 97.11% in treatment cells (T2, T3, T4, and T5, respectively), is used to bioremediate soil that has been contaminated with crude oil over the period of eight (8) weeks. In this experiment, brackish water was sprayed at four (4) different application rates (100, 200, 300, and 400 ml) with a high degradation effectiveness of above 90%. The TPAH results also revealed that 400 ml of brackish water decreased the TPH of contaminated soils up to 97.26%. This proved that the application of more brackish water during treatment T5 led to a more efficient breakdown of TPAH. An ANOVA was used to determine how brackish water affected TPAH concentration, and the results showed a significant difference between the treatment averages at 5% levels of significance. This demonstrates that there is a 95% possibility that the difference in treatment means was caused by a variation in the volume of brackish water applied.

Table 6: Mean Results of TPAH Concentrations (mg/kg) in Crude Oil Polluted Soil Remediated with Brackish Water

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	32.23	32.2	32.23	32.18	32.22
2	29.76	12.96	9.94	5.97	4.24
4	21.59	7.29	6.04	3.44	2.18
6	18.80	3.65	2.36	1.20	0.97
8	16.68	1.82	1.23	0.98	0.88

Table 7: Mean Results of TPAH Concentrations Reduction (%) in Brackish Water Remediated Soil

Period, weeks	Treatment				
	T1	T2	T3	T4	T5
0	0.00	0.00	0.00	0.00	0.00
2	7.66	59.76	69.17	81.46	86.85
4	33.01	77.37	81.25	89.31	93.25
6	41.67	88.67	92.69	96.28	96.99
8	48.25	94.34	96.20	96.96	97.26

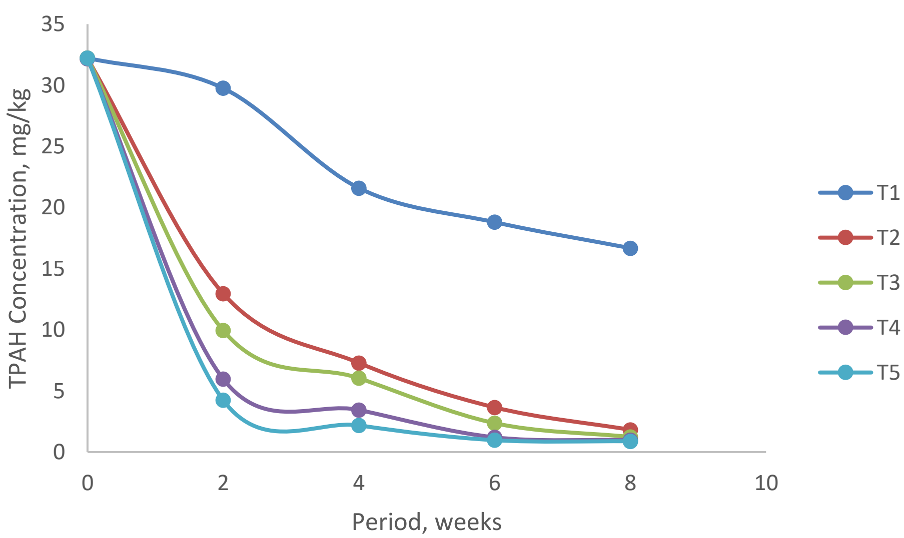

Fig. 3: Potential of Brackish Water on TPAH Degradation in Crude Contaminated Soil

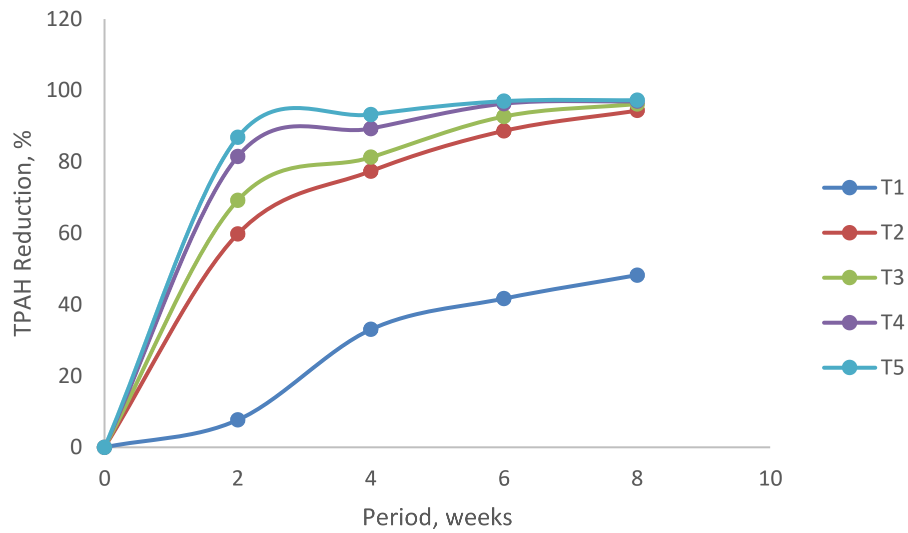

Fig. 4: TPAH Reduction on Influence of Brackish Water in Crude Contaminated Soil

3.4 Effect of THB on Crude Oil Contaminated Soil

Figure 5 illustrates the THB in the soil treated with various volumes of brackish water, which has a high concentration of nitrogen, phosphorus, and potassium. Because the cell (T5) with the highest volume of brackish water held the biggest population of THB, THB grew in the treatment cells with increased application rate. When brackish water was provided in varying amounts to the cells (T1, T2, T3, T4, and T5), the number of THB in each cell changed until it peaked (Table 8). The elimination of total petroleum hydrocarbon (TPH) concentration throughout treatment cells was attributed to changes in microbial activity, according to Ekemube *et al*. [10] findings. The growth was probably caused by the environment's great favoritism of the soil THB present there. As a result, as the experiment's time span came to an end, the number of THB decreased. This might be a result of the soil's altered by crude oil having fewer nutrients. As a result, the conclusions made by Chibuike *et al*. [16] are supported. The proper nutrients are an excellent method for boosting the metabolic activity of microorganisms, claim Onakaughoto *et al*. [17].

Table 8: Mean Results of THB Population (10^5cfu/mg) in Crude Oil Polluted Soil Remediated with Brackish

Period, weeks	Total Heterotrophic Bacteria, THB (10^5cfu/mg)				
	T1	T2	T3	T4	T5
0	3.47	3.47	3.42	3.51	3.4
2	3.74	7.38	8.38	8.55	8.9
4	3.83	9.6	10.74	11.72	12.21
6	2.68	5.67	6.18	6.34	6.71
8	1.88	4.7	5.13	5.42	5.47

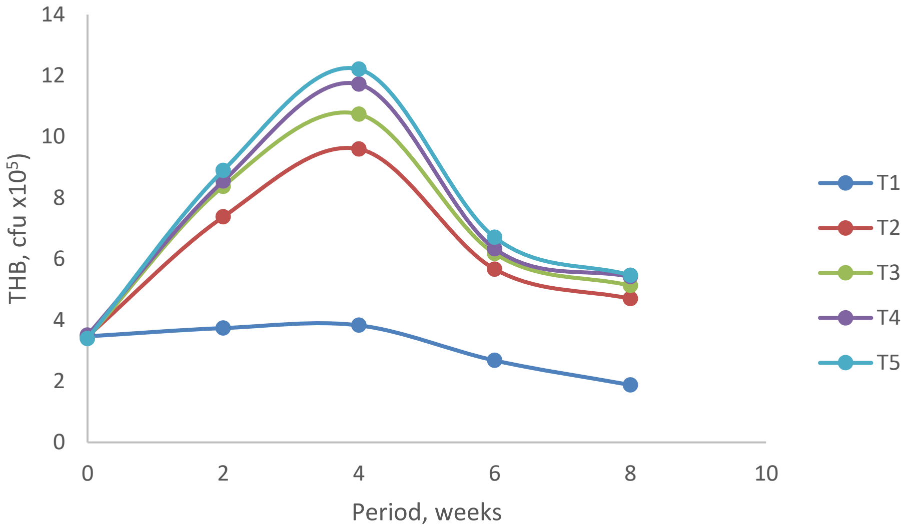

Fig. 5: Influence of Brackish Water on THB in Crude Oil Contaminated Soil

4 Conclusion

Using an investigative technique, the effectiveness of brackish water as biostimulant in the remediation of crude oil polluted soil was investigated. The laboratory analysis's test results support the following conclusions:

i. The United States Department of Agriculture's (USDA) textural classification of soil determined that the unpolluted area was a loamy sand soil type. Additionally, because brackish water contains a high concentration of NPK (6.21, 0.69, and 4.35 mg/kg, respectively), it has the capacity to clean up soil that has been contaminated with crude oil.

ii. Brackish water had an impact on the biodegradation of total petroleum hydrocarbon (TPH) in each of the crude oil-contaminated soil's cells (T1, T2, T3, T4, and T5). Its high nutritional composition (NPK) is the reason for this. After eight (8) weeks of remediation, the TPH of this brackish water has decreased to 94.73, 96.10, 97.45, and 98.73% for each treatment (T2, T3, T4, and T5).

iii. iii. In all of the cells (T1, T2, T3, T4, and T5) of the crude oil-contaminated soil, brackish water had an impact on the degradation of total petroleum hydrocarbons (TPAH). Its high nutritional composition (NPK) is the reason for this. At eight (8) weeks of remediation, the brackish water's TPAH has decreased to 94.04, 96.00, 96.80, and 97.11% in all treatment (T2, T3, T4, and T5, respectively).

iv. Due of brackish water's high nutrient content (NPK), total heterotrophic bacteria were impacted by the stimulant in the biodegradation of TPH and PAH in the crude oil-contaminated soil. Within eight (8) weeks of treatment, these THB start to decline as the stimulants start to diminish.

5 References

[1] Wanga, S., Xub, Y., Lin, Z., Zhang, J., Norbu, J., & Liu, W. (2017). The Harm of Petroleum Polluted Soil and its Remediation Research. AIP Conference Proceedings 1864, 020222 (2017); https://doi.org/10.1063/1.4993039 (Accessed on 20th January 2023).

[2] Ekemube, R. A., Emeka, C., Nweke, B., Atta, A. T., & Opurum, A. N. (2022). Evaluation of Simulated Petroleum Hydrocarbon on the Physicochemical Properties of Soil, *Research Journal of Ecology and Environmental Sciences 2*(4), 195 – 210.

[3] Shan, B. Q., Zhang, Y. T., Cao, Q. L., Kang, Z.Y., & Li, S.Y. (2014). Growth responses of six Leguminous Plants Adaptable in Northern Shaanxi to Petroleum Contaminated Soil. *Environmental Science, 35*(3), 1125 1130.

[4] Han, J. C. (2013). Introduction to Land Engineering. Beijing: Science Press, 188-191 (in Chinese)

[5] Zhang H R, Li P J, Sun T H, (2001). Bioremediation on 4 soils contaminated by petroleum oils using prepared bed processes. *Journal of Agro-Environment Science*, 20(2), 76-80.

[6] Thapa, B., Kumar, A., & Ghimire, A. (2012). A Review on Bioremediation of Petroleum Hydrocarbon Contaminants in Soil. *Kathmandu University Journal of Science, Engineering and Technology, 8*(1), 164-170.

[7] Hill, G. T., Mitkowski, N. A., Aldrich-Wolfe, L., Emele, L. R., Jurkonie, D. D., & Ficke, A. (2020). Methods for Assessing the Composition and Diversity of Soil Microbial Communities. *Apply Soil Ecology, 15*(2), 25

36.
[8] Liste, H. H., & Alexander, M. (2000). Plant Promoted Pyerne Degradation in Soil. *Chemosphere, 40*, 7 – 10
[9] Shen, T. M, Huang, G. Q., & Li, N, (2002). In-situ remediation technique for oil-polluted soil. *Environmental Science Trends*, (3): 13-15. Relevance. *Hindawi Scientifica*, *2019*, 1 – 10.
[10] Ekemube, R. A., Davis, D. D., Dumkhana, B. B., Amgbara, T. O. (2021). Combined Effects of Spent Mushroom Substrate (SMS) and NPK Fertilizer in the Remediation of Crude Oil Polluted Soil. *International Journal of Academic Engineering Research (IJAER), 5*(2), 22 – 27
[11] Nkakini, S. O., **Ekemube, R. A.**, & Igoni, A. H. (2019a). Development Of Predictive Model for Fuel Consumption During Ploughing Operation in Agricultural Soil. *European Journal of Engineering and Technology, 7*(1), 16 – 30.
[12] Nkakini, S. O., **Ekemube, R. A.**, & Igoni, A. H. (2019b). Modeling Fuel Consumption Rate for Harrowing Operations in Loamy Sand Soil. *European Journal of Agriculture and Forestry Research, 7*(2), 1 – 12.
[13] Igoni, A. H., **Ekemube, R. A.**, & Nkakini, S. O. (2019). Predicting Tractor Fuel Consumption during Ridging on a Sandy Loam Soil in a Humid Tropical Climate. *Journal of Engineering and Technology Research*, *11*(3), 29 - 40.
[14] Cowan, S.T. (1974). Cowan and Steel's Manual for Identification of Medical Bacteria, 2nd ed. Cambridge: university press.
[15] Buchanan, E., Gibbons, N., & Stewart, W. (1994). Bergeys. Manual of Determinative Bacteriology, Sol Energy, 24, Pp. 606-607.
[16] Chibuike, G. U., & Obiora, S. C. (2013). Bioremediation of Hydrocarbon – Polluted Soils for Improved Crop Performance. *International Journal of Environmental Sciences, 4*(3), 223 – 238.
[17] Onakughotor, E. D., & Agule, P. O. (2014). Impact of the Age of Particulates on the Bioremediation of Crude Oil Polluted Soil. *Journal of Applied Chemistry. 7*(11), 24 – 33.