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Effect of Varying E-Safe Concentrations on Physicochemical Properties and Hydrocarbon Remediation of Aged Crude Oil-Polluted Soil

[1]Iheagwam Kelechi Sigismund, [2] Azorji Johnpaul Nnawuike, [3]Emeka Joseph Emmanuel and [3]Uzowulu Ebere
[1]Department of Microbiology, Hezekiah University, Umudi, Imo State, Nigeria
[2]Department of Biotechnology, Hezekiah University, Umudi Imo State, Nigeria
[3]Department of Chemistry, Hezekiah University, Umudi, Imo State, Nigeria

ABSTRACT

Background and Objective: Crude oil contamination significantly alters the physicochemical properties of soil, posing long-term threats to soil fertility, microbial life, and food security, especially in developing countries like Nigeria. While various remediation products exist, region-specific and affordable solutions remain limited. This study aimed to evaluate the effectiveness of different concentrations of E-safe in remediating aged crude oil-impacted soil by assessing its impact on key physicochemical parameters and hydrocarbon degradation. **Materials and Methods:** A laboratory-based experiment was conducted using soil collected from a crude oil-contaminated site in Rivers State. The topsoil (0-30 cm) was sieved and artificially treated with five concentrations of E-safe (0, 1.5, 3, 6, and 12 mL/kg of soil) in a Completely Randomized Design. Each treatment was applied to 1 kg of soil and monitored for 8 weeks. Total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbons (PAH), pH, nitrate, and phosphate were analyzed using standard analytical procedures. Data were presented in charts and tables, with means separated using DMRT at $p<0.05$. **Results:** The E-safe significantly enhanced hydrocarbon degradation and improved soil conditions. The highest TPH degradation (98.79%) was observed at 12 mL/kg E-safe, while the control showed the least degradation (13.37%). The PAH removal ranged from 80.16% to 98.78% across treated groups. Soil pH shifted from slightly acidic to basic with increasing E-safe concentration. All E-safe treatments demonstrated over 60% improvement in degradation compared to the control. **Conclusion:** The E-safe effectively improved the remediation of aged oil-contaminated soils, particularly at higher concentrations. These findings support its potential use in developing countries where crude oil pollution is a persistent problem. Future studies should investigate long-term soil health and microbial recovery following E-safe application to refine optimal dosing and environmental safety.

KEYWORDS

E-safe, crude oil, soil, physicochemistry, total petroleum hydrocarbons (TPH)

INTRODUCTION

Crude oil contamination in the Niger Delta Region of Nigeria is gaining more prominence as a result of increased upstream and downstream activities of the petroleum industry[1]. The oil spillage emanating from petroleum tankers, product pipelines, petrol service stations, black market dealers in petroleum products,

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and illegal bunkering and crude oil theft are the major causes of oil pollution in Nigeria, especially on farmland, resulting in increased deleterious effects and perturbations of the natural ecology. These petroleum hydrocarbon release-induced ecological changes lead to adverse outcomes such as reduced crop yield, hence the need to use biological methods in cleaning up these crude oil spills so as to restore the region and farmland back to a good shape for farming[2].

The problem of crude oil pollution as an oil-producing country has drawn serious concern for a way out of it. Several methods have been employed to remove crude oil pollution from the soil, and some of them can be detrimental to the environment[3]. The use of microorganisms and their biosurfactant products to remediate hydrocarbon-polluted sites has been widely studied[4]. However, there are limitations on dependence on induced *in situ* biosurfactant production (due to inherently unpredictable *in situ* conditions) or industry-manufactured surfactants, which may have more deleterious ecological consequences than the contaminating hydrocarbon. The design and chemical nature of E-safe cleaning solution make it ideal and most promising for reversing Nigeria's and the global hydrocarbon pollution nightmare. The objective of this study was to evaluate the effectiveness of different concentrations of E-safe in enhancing the degradation of total petroleum hydrocarbons (TPH) and improving the physicochemical properties of aged crude oil-contaminated soil collected from Rivers State, Nigeria.

MATERIALS AND METHODS

Study area: The study was carried out from July to October, 2024 at the Teaching and Research Laboratory of the Department of Biological Sciences, Hezekiah University, Umudi, Imo State, located at Latitude 5.3866°N, and Longitude 6.9916°E.

Collection and processing of soil samples: Soil samples used in this study were collected from an aged oil-polluted site in Rivers State, Nigeria. The samples were collected by using an improvised soil auger at 0 cm to 30 cm. Approximately 4 kg of crude oil-polluted soil was measured and filled in plastic buckets and spaced 1.5 m apart. The buckets were perforated at the base to allow aeration. The E-safe product was thoroughly mixed and applied in each of the experimental soils at five treatment levels: 6, 12, 24, 48, and 0 mL (control). The set up was left for a period of 8 weeks with 72 hourly turning of the soil during the period before monitoring the rate of oil degradation in each of the concentrations applied. pH, phosphate and nitrate levels in the soil were also monitored for a period of four weeks[5].

Sample preparation: The samples were air-dried after being transported to the laboratory, and any grass or other items were removed manually. After rolling the samples to break up big clumps of soil particles, sifting was performed using a mechanical sieving system with various mesh sizes[6]. For further investigation, the sieved samples were placed in their respective cleaned and labeled plastic buckets.

Preliminary analysis of soil samples: Preliminary physicochemical properties of the crude oil-contaminated soil were carried out to ascertain the initial level of TPH, pH, nitrate, and phosphate.

Soil pH determination: To determine pH, the Mettler Toledo Seven Easy pH meter and the Walkley-Black Titrimetric method were used[7]. Determination of phosphate and nitrate content of soil samples. Nitrate and phosphate levels in test soil samples were, respectively determined by spectrophotometric methods 8171 and 8048, using the portable DR 900 multiparameter spectrophotometer[8] and Iheagwam *et al.*[9]

Determination of TPH concentration of soil samples: The TPH content of the soil samples was extracted[10]. About 10 g of the soil sample was carefully mixed with 150 mL of dichloromethane, which was used as the extraction solvent, and extracted for 4 hrs and 30 min. This was done in the presence of 2.5 g of dried sodium sulfate and 300 µg/mL of 1-chloro-octadecane as a surrogate standard. 0.3 g of silica was introduced into the extraction mixture after the extraction to facilitate the adsorption of polar materials like animal fats and oils from vegetable materials. The extracts were later passed through a Whatman glass fiber filter for filtration. The separation and determination of TPH contained in the soil samples were carried out with Gas Chromatography equipped with Flame Ionization Detector (GC-FID) (Agilent 6890N). A concentrated 1 µL of the sample was introduced into the GC column with a micro-syringe previously rinsed with dichloromethane (blank) and the sample. The TPH was determined at a specific chromatogram in ppm.

Statistical analysis: Data collected were presented in charts and tables, and means were separated using Duncan's Multiple Range Test (DMRT) at a probability of <0.05 level.

RESULTS AND DISCUSSION

TPH, PAH, and physico-chemical parameters: The total petroleum hydrocarbon (TPH), polyaromatic hydrocarbon (PAH) degradation, and physico-chemical results of the various treatment regimes in the E-safe bioremediation enhancement efficacy testing are shown in Table 1.

Changes in the physicochemical indices for the bioremediation study control sample: The results of the changes in the bioremediation indices (TPH, PAH, THB, THUB, THUB/THB ratio, pH, nitrate, and phosphate) for the control samples are shown in Table 1 respectively. The changes indicate a slight reduction in the concentration of TPH from 21701.67 mg/kg to 21422.65 mg/kg on the 2nd week of the study. The TPH of the sample further reduced from 21201.76 mg/kg on the 4th week to 18899.07 mg/kg on the 8th week of the study. The contaminated soil had a total hydrocarbon-utilizing bacteria population to Heterotrophic bacterial ratio that indicated evidence of a hydrocarbon clastic environment. This is evidenced by the THUB/THB ration that exceeded 1% at the initial measurement with values that ranged from 3.38% at the start of the experiment to a maximum ratio of 8.36% attained by the 4th week then a drop to 7.87 and 6.94%, respectively for the 6th and 8th weeks was recorded indicating the onset of the death phase of the growth curve. The pH of the experimental setup was observed to remain within the range 6.14 to 6.33 for 8 weeks. The nitrate concentration of the sample was observed to slightly decline from 25.76 mg/kg to 21.77 mg/kg on the 8th week. The phosphate concentration declined slightly from 0.54 mg/kg on the initial day to 0.53 mg/kg for weeks 1 and 2 of the experimental setup, then to 0.49 mg/kg.

Changes in the physicochemical indices for the bioremediation study, 1.5 mL E-safe/kg treated soil: The result presented in Table 2 showed the changes in the bioremediation indices for the 1.5 mL/kg E-safe treatment. The changes showed that there was a slight reduction in the concentration of TPH from 21701.67 mg/kg to 18542 mg/kg on the 2nd week of the study. The TPH values from the initial week to the 4th week were 21701.67 mg/kg to 15435.45 mg/kg before a steady decline, for which the concentration fell to 6943.48 mg/kg on the 8th week of the study. The PAH column of Table 2 indicates a corresponding but slight decline in the concentration as the value reduced from 15308.37 mg/kg to 10256 mg/kg on the 2nd week of study. The PAH of the sample further reduced from 8468.92 mg/kg on the 4th week to 3037.32 mg/kg on the 8th week of the study. The changes and interaction between the total heterotrophic bacteria (THB) and total hydrocarbon utilizing bacteria (THUB) count 1. The findings showed the increase in the total heterotrophic bacteria (THB) count as the growth went from a lag phase, as its count went from 6.3×10^5 CFU/g, thereafter the growth peaked into an exponential phase of 7.1×10^5 to 8.2×10^6 CFU/g within the week 1 and week 2 after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred. Furthermore, the total hydrocarbon utilizing bacteria (THUB) population increased as the growth went from a lag phase, as its count went from 2.13×10^4 CFU/g, thereafter the growth peaked into an exponential phase of 6.5×10^4 to 9.7×10^5 CFU/g within the week 1 and week 2, after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred.

Tabe 1: TPH, PAH, microbiological and physico-chemical parameters

	TPH	PAH	THB	THUB			NO_3^-	PO_4^-
Time	mg/kg		Cfu/g		THUB/HUB (%)	pH	mg/kg	
WKS Initial (SD)	21701.67	15308.37	6.30×10^5	2.13×10^4	3.38	6 .14	25.76	0.54
1WKS (SD)	21651.23	15128.15	6.60×10^5	2.40×10^4	3.64	6.28	24.93	0.53
2WKS (SD)	21422.65	15001.84	6.90×10^6	4.90×10^5	7.10	6.33	24.63	0.53
4WKS (SD)	21201.76	14915.04	6.70×10^6	5.60×10^5	8.36	6.33	24.49	0.52
6WKS (SD)	19590.63	13741.02	6.10×10^6	4.80×10^5	7.87	6.3	23.83	0.51
8WKS (SD)	18800.07	13642.23	3.60×10^6	4.30×10^5	6.94	6.33	21.77	0.49

Table 2: Results of physico-chemical parameters, TPH, PAH, and microbiological measurements in 1.5 mL E-safe/kg treated soil

	TPH	PAH	THB	THUB			NO_3^-	PO_4^-
Time	mg/kg		Cfu/g		THUB/HUB (%)	pH	mg/kg	
0WKS	21701.67	15308.37	6.30×10^5	2.13×10^4	3.38	6.14	25.76	0.54
1WKS	20250	12200	7.10×10^5	6.50×10^4	9.15	6.16	22.94	0.50
2WKS	18542	10256	8.20×10^6	9.70×10^5	11.83	6.19	22.1	0.46
4WKS	15435.45	8468.92	8.60×10^6	1.20×10^6	13.95	6.2	21.04	0.39
6WKS	10615.17	6324.91	8.10×10^6	8.40×10^5	10.37	6.31	17.38	0.26
8WKS	6943.48	3037.32	6.80×10^6	7.20×10^5	10.59	6.48	13.93	0.18

Table 3: Results of physico-chemical parameters, TPH, PAH, and microbiological measurements in 3 mL E-safe/kg treated soil

	TPH	PAH	THB	THUB			NO_3^-	PO_4^-
Time	mg/kg		Cfu/g		THUB/HUB (%)	pH	mg/kg	
0WKS	21701.67	15308.37	6.30×10^5	2.13×10^4	3.38	6.14	25.76	0.54
1WKS	15602	12450.12	3.68×10^6	3.20×10^5	8.70	6.23	20.12	0.41
2WKS	9650	9560.35	6.10×10^7	2.80×10^7	45.90	6.31	17.34	0.33
4WKS	7287.88	5799.64	5.90×10^7	2.80×10^7	47.46	6.38	14.83	0.21
6WKS	5342.32	3659.25	5.15×10^7	2.20×10^7	42.72	6.42	11.64	0.14
8WKS	3195.02	1458.21	4.65×10^7	1.80×10^7	38.71	6.53	6.86	0.10

The pH of the 1.5 mL/kg E-safe treated experimental setup was observed to remain within the range between 6.14 to 6.48 for 8 weeks. The nitrate concentration of the sample was observed to slightly decline from 25.76 mg/kg to 13.93 mg/kg on the 8th week. The phosphate concentration declined slightly from 0.54 mg/kg on the initial day to the 0.18 mg/kg for the 8 weeks of the experimental setup.

Changes in the physicochemical indices for the bioremediation study using 3 mL E-safe/kg soil treatment: The result presented in Table 3 shows the changes in the bioremediation indices for the 3 mL E-safe/kg soil treatment. The changes show that there was a slight reduction in the concentration of TPH from 21701.67 mg/kg to 9650 mg/kg on the 2nd week of the study. The TPH values from the initial week to the 4th week were 21701.67 mg/kg to 7287.88 mg/kg before a steady decline, for which the concentration fell to 3195.02 mg/kg on the 8th week of the study. The PAH column of Table 3 indicates a corresponding but slight decline in the concentration as the value reduced from 15308.37 mg/kg to 9560.35 mg/kg on the 2nd week of study. The PAH of the sample further reduced from 5799.64 mg/kg on the 4th week to 1458.21 mg/kg on the 8th week of the study. The findings showed the increase in the total heterotrophic bacteria (THB) count as the growth went from a lag phase, as its count went from 6.3×10^5 Cfu/g, thereafter the growth peaked into an exponential phase of 3.68×10^6 to 6.1×10^7 Cfu/g within the week 1 and week 2 after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred. Furthermore, the total hydrocarbon utilizing bacteria (THUB) population increased as the growth went from a lag phase, as its count went from 2.13×10^4 Cfu/g, thereafter the growth peaked into an exponential phase of 3.2×10^5 to 2.8×10^7 Cfu/g within the week 1 and week 2, after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred. The pH of the experimental setup was observed to remain within the

Table 4: Results of physico-chemical parameters, TPH, PAH and microbiological measurements in 6 mL E-safe/kg treated soil

	TPH	PAH	THB	THUB			NO_3^-	PO_4^-
Time	mg/kg		Cfu/g		THUB/HUB (%)	pH	mg/kg	
0WKS	21701.67	15308.37	6.30×10^5	2.13×10^4	3.38	6.14	25.76	0.49
1WKS	13520.05	9554.01	3.30×10^6	6.30×10^5	19.09	6.23	20.34	0.42
2WKS	7422.23	5687.65	8.50×10^7	4.80×10^7	56.47	6.38	13.54	0.32
4WKS	5287.88	3770.38	9.80×10^7	7.20×10^7	73.47	6.51	9.36	0.21
6WKS	3258.18	2136.03	8.60×10^7	6.10×10^7	70.93	6.58	7.11	0.14
8WKS	1794.87	776.68	6.10×10^7	4.30×10^7	70.49	6.67	4.31	0.10

Table 5: Results of physico-chemical parameters, TPH, PAH, and microbiological measurements in 12 mL E-safe/kg treated soil

	TPH	PAH	THB	THUB			NO_3^-	PO_4^-
Time	mg/kg		Cfu/g		THUB/HUB (%)	pH	mg/kg	
0WKS	21701.67	15308.37	6.30×10^5	2.13×10^4	3.38	6.14	25.76	0.49
1WKS	9566.25	7254.36	3.60×10^6	1.10×10^6	30.56	6.23	17.63	0.38
2WKS	5875.32	4156.71	7.90×10^7	4.80×10^7	60.76	6.48	9.15	0.27
4WKS	2515.18	2077.2	8.10×10^7	7.20×10^7	88.89	6.74	4.97	0.13
6WKS	1088.79	1080.86	7.30×10^7	5.50×10^7	75.34	6.79	2.67	0.12
8WKS	265.0992	153.23	6.30×10^7	4.25×10^7	67.46	6.84	0.84	0.04

ranges between 6.14 to 6.53 for 8 weeks. The nitrate concentration of the control samples was observed to slightly decline from 25.76 to 14.83 mg/kg on the 4th week. The phosphate concentration declined slightly from 0.54 mg/kg on the initial day to the 0.53 mg/kg for the weeks 1 and 2 of the experimental setup and to 0.10 on the 8th week.

The result presented in Table 4 shows the changes in the bioremediation indices for the 6 mL/kg treated soil. The changes show that there was a slight reduction in the concentration of TPH from 21701.67 mg/kg to 7422 mg/kg in the 2nd week of the study. The TPH values from the initial week to the 4th week were 21701.67 mg/kg to 5287.88 mg/kg before a steady decline, for which the concentration fell to 1794.87 mg/kg on the 8th week of the study. The PAH column of Table 4 indicates a corresponding but slight decline in the concentration as the value reduced from 15308.37 mg/kg to 5687.65 mg/kg in the 2nd week of study. The PAH of the sample further reduced from 3770.38 mg/kg on the 4th week to 776.68 mg/kg on the 8th week of the study.

The changes and interaction between the total heterotrophic bacteria (THB) and total hydrocarbon utilizing bacteria (THUB) count were presented in Table. 4. The findings showed the increase in the total heterotrophic bacteria (THB) count as the growth went from a lag phase, as its count went from 6.3×10^5 Cfu/g, thereafter the growth peaked into an exponential phase of 8.5×10^7 to 9.8×10^7 Cfu/g within the week 2 and week 4 after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred. Furthermore, the total hydrocarbon utilizing bacteria (THUB) population increased as the growth went from a lag phase, as its count went from 2.13×10^4 Cfu/g, thereafter the growth peaked into an exponential phase of 6.3×10^5 to 7.2×10^7 Cfu/g within the week 1 and week 4, after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred. The pH of the experimental setup was observed to remain within the ranges between 6.14 to 6.67 for 8 weeks. The nitrate concentration of the samples was observed to slightly decline from 25.76 to 4.31 mg/kg on the 4th week . The phosphate concentration declined slightly from 0.49 mg/kg on the initial day to the 0.32 mg/kg for the weeks 1 and 2.

Changes in the physicochemical indices for the bioremediation study using 12 mL/kg treated soil: The result presented in Table 5 shows the changes in the bioremediation indices for the 12 mL/kg treated soil. The changes show that there was a slight reduction in the concentration of TPH from 21701.67 mg/kg to 5875.32 mg/kg on the 2nd week of the study. The TPH values from the initial week to the 4th week was 21701.67 mg/kg to 2515.18 mg/kg mg/kg before a steady decline, for which the concentration fell to

265.0992 mg/kg on the 8th week of the study. The PAH column of Table 5 indicates a corresponding but slight decline in the concentration as the value reduced from 15308.37 mg/kg to 4156.71 mg/kg on the 2nd week of study. The PAH of the sample further reduced from 2077.2 mg/kg on the 4th week to 153.23 mg/kg on the 8th week of the study. The findings showed the increase in the total heterotrophic bacteria (THB) count as the growth went from a lag phase, as its count went from 6.3×10^5 Cfu/g, thereafter the growth peaked into an exponential phase of 3.6×10^6 to 7.9×10^7 Cfu/g within the week 1 and week 2 after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred. Furthermore, the Total Hydrocarbon Utilizing Bacteria (THUB) population increased as the growth went from a lag phase, as its count went from 2.13×10^4 Cfu/g, thereafter the growth peaked into an exponential phase of 4.8×10^7 to 7.2×10^7 Cfu/g within the week 2 and week 4, after which the death phase was encountered from the 6th week to 8th week as the microbial population deterred. The pH of the experimental setup was observed to remain within the ranges of 6.14 to 6.84 for 8 weeks. The nitrate concentration of the sample was observed to slightly decline from 25.76 mg/kg to 0.84 mg/kg on the 8th week. The phosphate concentration declined slightly from 0.49 mg/kg on the initial day to 0.27 mg/kg for the weeks 1 to 3 of the experimental setup, after which it fell to 0.04 mg/kg.

E-safe enhances the rate of hydrocarbon biodegradation[11]. Different parameters of the test soil samples were measured at various treatment levels over a period of 8 weeks. The phosphate concentration in the soil samples also showed significant differences ($p<0.05$) in the means at various concentrations. However, the concentration in the soil samples showed a decrease as the treatment levels increased, which is in line with the opinion of researchers[12-14], respectively.

Accordingly, the maximum TPH concentration was observed in the control. The oil removal rates were within the ranges of 13.37- 98.79% following the treatment with E-safe and 8 weeks of aeration. The TPH concentrations decreased substantially after 8 weeks of treatment in the laboratory. About 98.79% TPH degradation was achieved at 12 mL E-safe/kg soil, followed by 6 mL E-safe/kg soil (91.73%), while the least percentage removal of TPH was recorded in the control (13.37%). The results of the study further revealed that more than 90% of the hydrocarbon-contaminated soil was recovered by treatment with E-safe product at 12 mL E-safe/kg soil, 6 mL E-safe/kg soil, 3 mL E-safe/kg soil, and 1.5 mL E-safe/kg soil, respectively. The TPH concentrations decreased substantially after 8 weeks of treatment in a dose-dependent manner[15].

CONCLUSION

This study demonstrates that E-safe is an effective and low-cost solution for the bioremediation of aged crude oil-contaminated soils, achieving up to 98.79% TPH degradation within 8 weeks. Its application significantly improved soil physicochemical properties, making it a practical option for environmental cleanup in oil-polluted regions. Adoption of E-safe by industries and government agencies could offer a rapid and efficient approach to mitigating hydrocarbon pollution. Further research is recommended to assess its efficacy in deeper soil layers and groundwater systems.

SIGNIFICANCE STATEMENT

Crude oil pollution remains a critical environmental issue, particularly in developing countries where affordable and effective remediation options are limited. This study highlights the potential of E-safe, a low-cost bioremediation product, in significantly enhancing the degradation of petroleum hydrocarbons in contaminated soils. With up to 98.79% TPH removal observed within 8 weeks, E-safe offers a practical and scalable solution for restoring soil health and reducing ecological risks associated with crude oil pollution. This study will help researchers uncover critical areas of soil bioremediation and microbial recovery that remain underexplored. Thus, a new theory on region-specific, cost-effective bioremediation strategies for hydrocarbon-polluted environments may be developed.

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