Efficacy of E-Safe Product in Decontamination of Hydrocarbon Contaminated Soil

Iheagwam, K.S.[1], Uzowulu, O.E.[1], Azorji, J.N.[2], Emeka, J.E.[3] & Effiong, N.E.[1]
[1]Department of Microbiology, Hezekiah University, Umudi, Imo State
[2]Department of Biological Sciences, Hezekiah University, Umudi, Imo State
[3]Department of Chemical Sciences, Hezekiah University, Umudi, Imo State
Corresponding author: jnazorji17@gmail.com

ABSTRACT: *Effectiveness of E-safe© product in the recovery of soil contaminated with total petroleum hydrocarbon (TPH) was investigated at the Teaching and Research Laboratory of the Department of Microbiology, Hezekiah University, Umudi, Imo State. The study was conducted on soil sample collected from aged crude oil polluted site in Rivers State.The top soil (0-30cm) was sieved using a 5mm mesh to remove gravel and debris prior to analysis. The pH, Phosphate, Nitrate, and TPH levels in the soil were analyzed using well established standard analytical protocols. E-safe product was artificially spiked in each of the 4kg soil at 0 ml, 6 ml, 12 ml, 24 ml, and 48 ml and arranged in a Completely Randomized Design. The percentage degradation of TPH in soil was monitored for a period of four (4) weeks. Results obtained showed that the soil pH was slightly acidic to basic. The findings showed Nitrate (4.97 – 24.42 ppm) and phosphate (0.134 – 0.53 ppm) ranges for both treated and control soils within the period. About 88.41% TPH degradation was achieved at 48 ml followed by 24 ml (75.63%) while the least percentage removal of TPH was recorded in the control (2.30%). The study further showed that soils contaminated with petroleum hydrocarbon altered soil physicochemistry, but more than 50% of the polluted soils were recovered by treatment with E-safe product at 48 ml, 24 ml and 12 ml respectively. The mineral components of the E-safe product could have acted as effective substance in the total removal of oil contaminants in the contaminated soil.*

KEYWORDS: Biodegradation, TPH, soil, E-safe, Imo State.

INTRODUCTION

Environmental contamination resulting from petroleum hydrocarbons (PHs) has occurred since ancient time naturally, but in the latest years, anthropogenic oil spills have become common [1]. Crude oil (petroleum) hydrocarbons are one of the most common groups of persistent organic pollutants [2–3]. Petroleum hydrocarbons have been implicated to be toxic to many living organisms due to their mutagenic and carcinogenic properties [4]. The low rate of decomposition

of oil and oil products in the environment triggers their accumulation and a gradual increase in their concentration in the environmental objects, including the soil [5].According to [6] globally, crude oil constitutes 33.5% of the energy requirements for human development and the bulk of this is obtained from drilling activities either offshore or onland. Paramount among the problems associated with drilling for crude oil is pollution including soil contamination [7]. It has been established that crude oil pollution occurs whenever unrefined hydrocarbons spill into the environment where it is exploited, explored or handled [8]. Pollution is an undesirable change in the physical, chemical, and biological characteristics of all the components of an environment, which can threaten human health and that of beneficial organisms [9]. Whenever a spill occurs, the immediate natural environment which is affected may include farmlands, surface water bodies (eg. fish ponds, and lakes) and overtime, groundwater through leachates and ultimately also the ambient atmosphere becomes polluted with it through volatilization [10].

[11] reported that crude oil pollution spells far more hazardous consequences for soil and soil borne organisms than air pollution because whatever is absorbed by plants would be rich in the content of water soluble constituents of petroleum oil some of which are toxic to plants. Crude petroleum oil production and its potential spillage have been reported to alter the carbon-nitrogen ratio and lead to nitrogen deficiency which can in turn threaten the survival of soil biota [3; 12]. The presence of crude in pore spaces in soil has been reported to expel air, deplete oxygen reserves and impede all forms of gaseous exchange between the atmosphere and soil thus limiting the survival of soil biocene as they (biocene) will lack elements essential for their growth and development [4].

These developments amongst others have heightened the growing quest by man to find solutions to crude oil polluted environments with efficiency and effectiveness, resulting in the search for the most appropriate remediation method(s)/technique(s) to adopt.Conventional oil spill countermeasures include various physical, chemical, and biological methods.Commonly used physical methods include booming and skimming, manual removal (wiping), mechanical removal, water flushing, sediment relocation, and tilling. Physical containment and recovery of bulk or free oil is the primary response option of choice in the United States for the cleanup of oil spills in marine and freshwater shoreline environments [13]. Chemical methods, particularly dispersants, have been routinely used in many countries as a response option [14]. However, chemical methods have not been extensively used in the United States due to the disagreement about their effectiveness and the concerns of their toxicity and long-term environmental effects [15]. With the recent development of less toxic chemical dispersants, the potential for their applications may increase. The other clean-up measures include the injection of gaseous oxygen into anaerobic zone of contaminated environment to stimulate biodegradation soil vent, or sparging, and the use of more soluble electron acceptors such as nitrates or sulphates. However, oxidation was reported to be slow and hydrocarbon degradation incomplete [6].

Although conventional methods, such as physical removal, often are the first response option, they rarely achieve complete cleanup of oil spills [16].Bioremediation is beginning to emerge as a

promising technology, particularly as a secondary treatment option for oil cleanup. Bioremediation has been defined as "the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes" [17]. It is an evolving method with the advantages of cost effectiveness and noninvasiveness to the species that are adapted to the environment. This technology is based on the premise that a large percentage of oil components are readily biodegradable in nature [7]. Bioremediation has several potential advantages over conventional technologies, such as being less costly, less intrusive to the contaminated site, and more environmentally benign in terms of its end products.As opined by [18] the success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment. Numerous scientific review articles have covered various factors that influence the rate of oil biodegradation [5; 9].

Among the more soluble and toxic compounds found in crude oil polluted soil and water, aromatic compounds (e.g., benzene, toluene, ethylbenzene, xylene isomers, and phenols) are of great concern as the removal of these compounds is extremely strenuous. Furthermore, they are highly toxic, which hinders the direct use of biological treatments [19]. To overcome this worrisome limitation, E-Safe has been introduced and applied in this research work for the reduction of clean-up costs and permanent elimination of hazardous wastes without the need for secondary clean-up. Therefore, the prime objective of the current investigation was the evaluation of effectiveness of various concentrations of E-safe on hydrocarbon contaminated soil.

MATERIALS AND METHODS

Study area
The study was carried out at the Teaching and Research Laboratory of the Department of Microbiology, Hezekiah University, Umudi, Imo State located at Latitude 5.3866° N, and Longitude 6.9916° E.

Collection of soil samples
Soil samples used in this study was collected from an aged oil polluted site in Rivers State, Nigeria. The samples were collected by using improvised soil auger at from 0 cm to 30 cm. The auger was screwed to the desired depth and the sample was withdrawn. Soil samples were transferred to plastic bags and sent to the laboratory for further studies. The soil has been polluted with Petroleum Hydrocarbons due to accidentalspills but more by third partyinterference on the crude oil pipelines.

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. 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 was carried out to ascertain the initial level of TPH, pH, Nitrate and Phosphate.

Soil pH Determination
To determine pH, Mettler Toledo Seven Easy pH meter and Walkley-Black Titrimetric method respectively were used (Oluseyi*et al*. 2014).

Determination of Phosphate and Nitratecontent 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 multi-parameterspectrophotometer following the method Yahaya *et al*., (2022) and Nwachukwu*et al*., (2019).

Experimental Design and treatment combination
The experimental design used in this experiment was Complete Randomized Design (CRD), with 5 treatments and 3 replicates. The total number of experimental soil samples (n) as the product of the number of treatments (T) and the number of replications (r); that is, n = rT . n = 3 x 5= 15. Approximately 4kg 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 soil at five treatment levels: 6 ml, 12 ml, 24 ml, 48 ml, and 0 ml (control) respectively. The set up was left for a period of four (4) weeks with 72 hourly turning of the soil during the period before monitoring the rate of oil degradation in each of the concentration applied. pH, Phospahte and Nitrate levels in the soil were also monitored for a period of four weeks.

Determination of TPH concentration of soil samples
The TPH content of the soil samples was extracted according to the methods described by previous researchers (Ibe et al., 2021; Yahaya *et al*., 2020; Ogoko, 2014). About 10 g of the soil sample was carefully mixed with 150 mL dichloromethane which was used as the extraction solvent and extracted for 4 hours 30 minutes. 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 oil 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.

Estimation of TPH degradation in the E-Safe treated soil samples

The proof of the TPH degradation effectiveness in each treatment level was evaluated by the gradual decline of the hydrocarbons and calculated by its removal efficiency using the following equation as reported by Yahaya *et al*., (2022):

Removal percentage $= \dfrac{C0 - Ce}{C0}$

Where,

C_0 and C_e represent the initial and final TPH concentrations (ppm), respectively.

Statistical analysis

Data collected were presented in charts and tables and means were separated using Duncan Multiple Range Test (DMRT) at probability of < 0.05 level.

RESULTS AND DISCUSSION

Figure 1 displays soil test result of measured parameters at various treatment levels over a period of 4 weeks.As shown in figure 1, values of pH highlight a general acidic to slightly basic soil. pH, NO_3 and PO_4 measured ranged as follows: $6.20 – 6.74$; $4.97 – 24.42$ and $0.134 – 0.53$ mg/kg respectively.pH among other variables in the soil could have been reported to control the circulation and enrichment of heavy metals in soil [20].The pH of soils in the experimental soil sample used in this study was slightly higher than the levels reported in the Niger Delta region of Nigeria [21] but aligns with the values reported by [22].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.
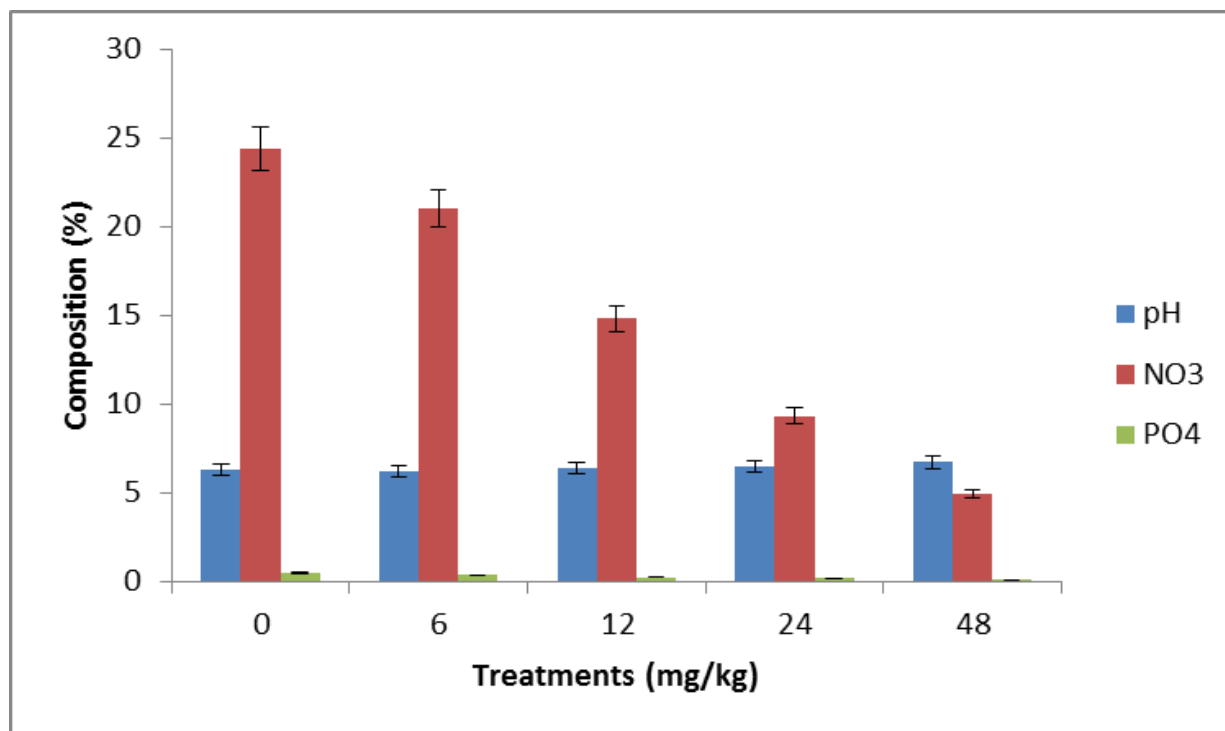
Figure 1: Preliminary soil sample exposed to different concentrations of E-safe

Table 1 shows the initial TPH values of experimental soil andtheir corresponding removal rates after the E-Safe treatmentand aeration at various concentrations.Accordingly, the maximum TPH concentration was observed in the control. The oil removal rates were within the ranges of 2.30-88.41% following the treatment with E-Safe and 4 weeks of aeration.TheTPHconcentrations decreased substantially after 4 weeks of treatment in the laboratory.As shown in **Table 1,**about 88.41% TPH degradation was achieved at 48 ml followed by 24 ml (75.63%) while the least percentage removal of TPH was recorded in the control (2.30%). The results of the study further revealed that more than 50% of the hydrocarbon contaminated soil was recovered by treatment with E-safe product at 48 ml, 24 ml and 12 ml respectively. Figures 2 to 6 shows the chromatograms indicating rate of degradation in the soil sample per treatment after 4 weeks of exposure.As is shown in Figures 2-6, theTPHconcentrations decreased substantially after 4 weeks of treatment in a dose-dependent manner.

Table 1: Concentration of Total Hydrocarbons at Initial Stage in Samples and percentage degraded after 4 weeks of exposure to E-safe

Treatments . (ml)	Initial TPH Conc.(ppm)	Residual (ppm)	%Degraded
0	21,701.67	21,201.76	2.30
6	21,701.67	15,435.45	28.87
12	21,701.67	9,495.58	56.24
24	21,701.67	5,287.88	75.63
48	21,701.67	2,515.18	88.41

Legend: TPH = Total Petroleum Hydrocarbon; Conc. = Concentration.

```
Injection Date  : 17/08/2023 10:42:17 AM
Sample Name     : Sandy+Clay soil              Location   : Vial 1
Acq. operator   : ARAL565                            Inj  : 1
                                            Inj Volume : 1 µl

Method          : C:/HPCHEM/1/METHODS/TPH-2.M
Last Changed    : 17/04/2023 09:51:36 AM by ARAL565
```
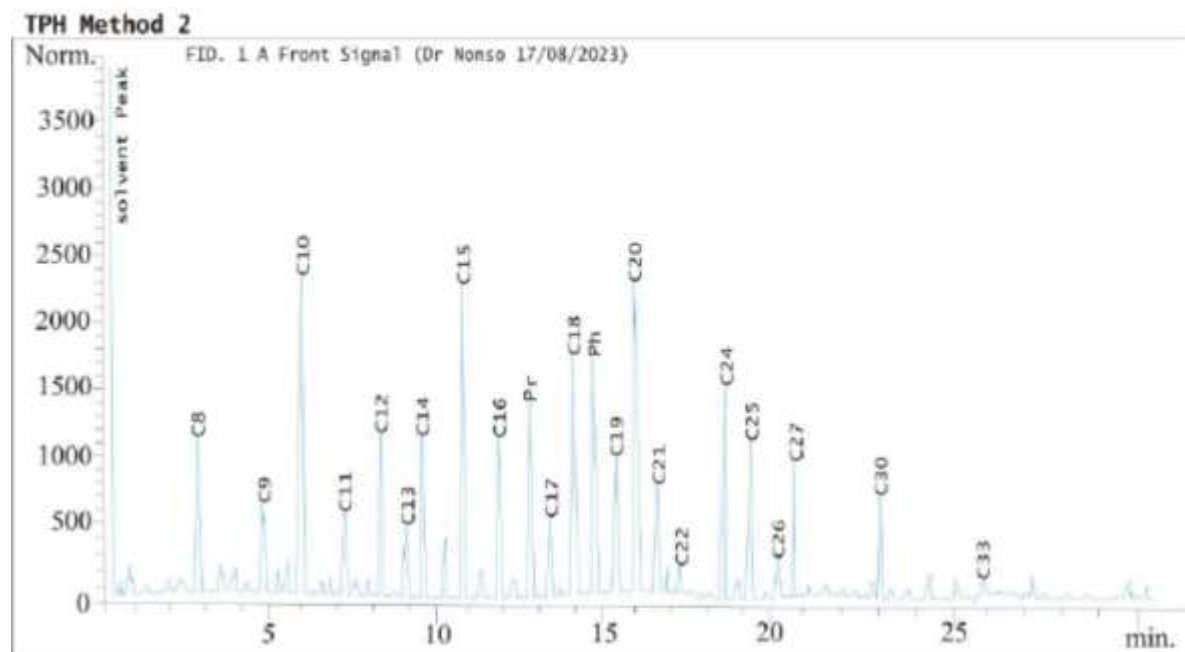

Figure 2: Initial TPH Concentration of test soil

Figure 2: TPH Concentration after 4 weeks with 0ml E-Safe

Figure 3: TPH Concentration after 4 weeks with 6ml E-Safe

```
Injection Date  : 17/08/2023 11:27:40 AM
Sample Name     : Sample 12                    Location   : Vial 4
Acq. operator   : ARAL565                          Inj   : 1
                                               Inj Volume : 1 µl

Method          : C:/HPCHEM/1/METHODS/TPH-2.M
Last Changed    : 17/04/2023 09:51:36 AM by ARAL565
```
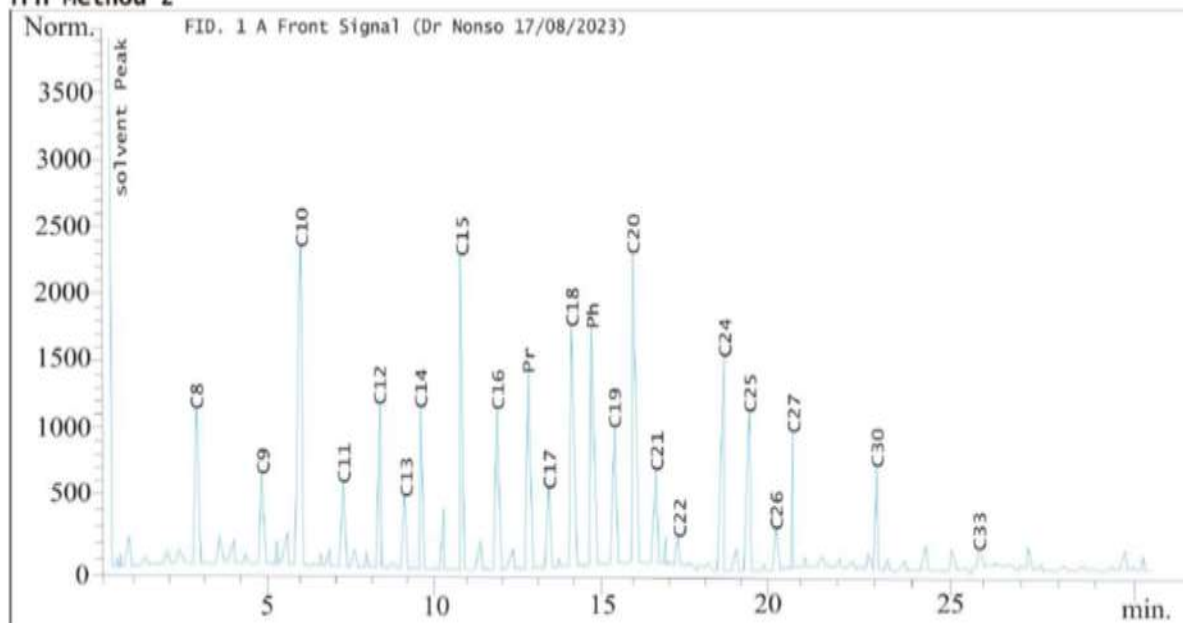

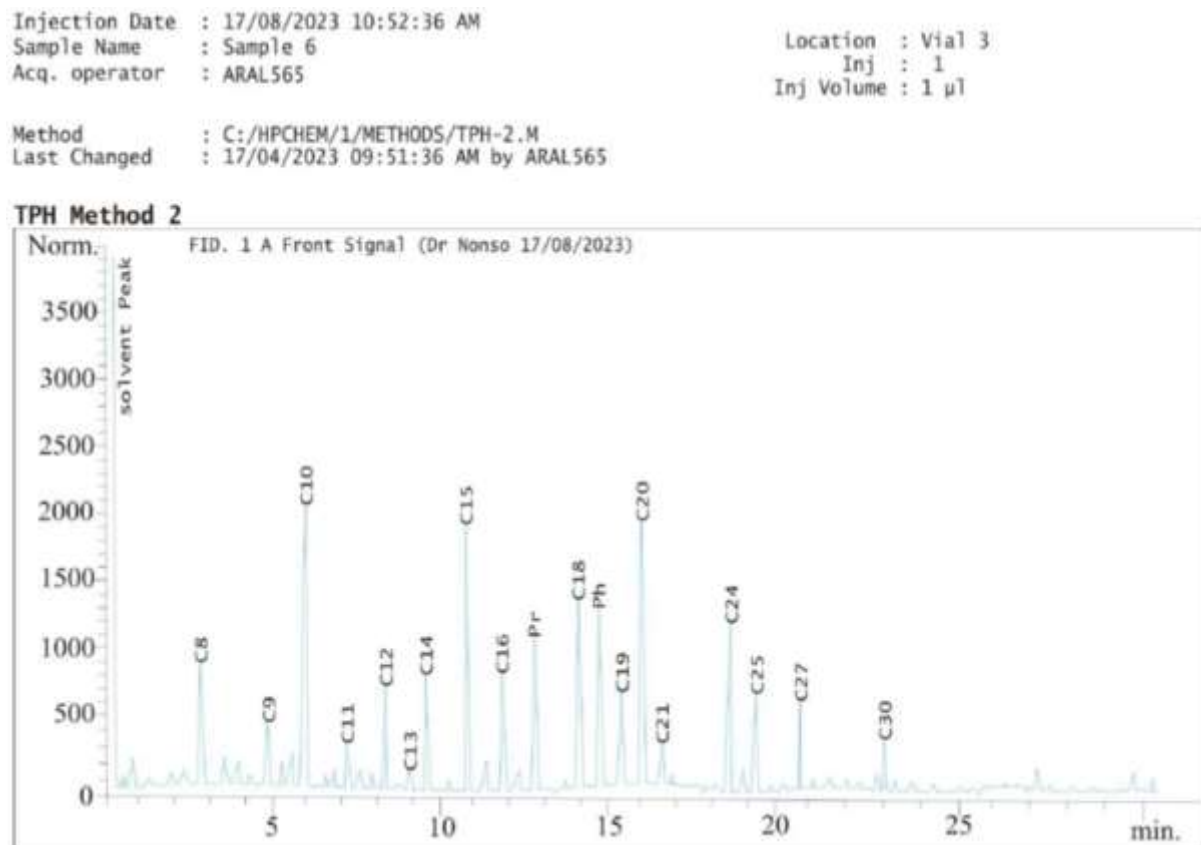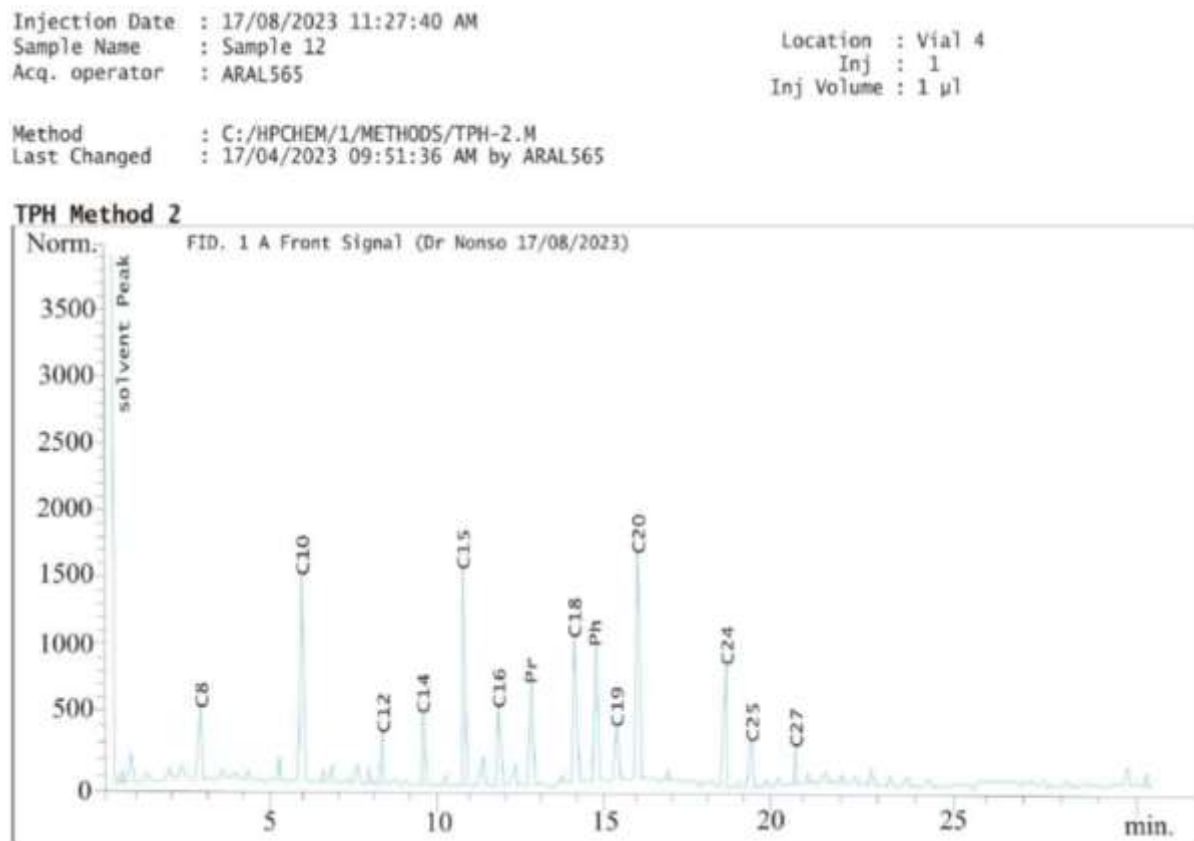**Figure 4**: TPH Concentration after 4 weeks with 12ml E-Safe

Injection Date : 17/08/2023 12:02:19 PM
Sample Name   : Sample 24   Location : Vial 5
Acq. operator   : ARAL565   Inj   : 1
                                               Inj Volume : 1 µl

Method   : C:/HPCHEM/1/METHODS/TPH-2.M
Last Changed   : 17/04/2023 09:51:36 AM by ARAL565
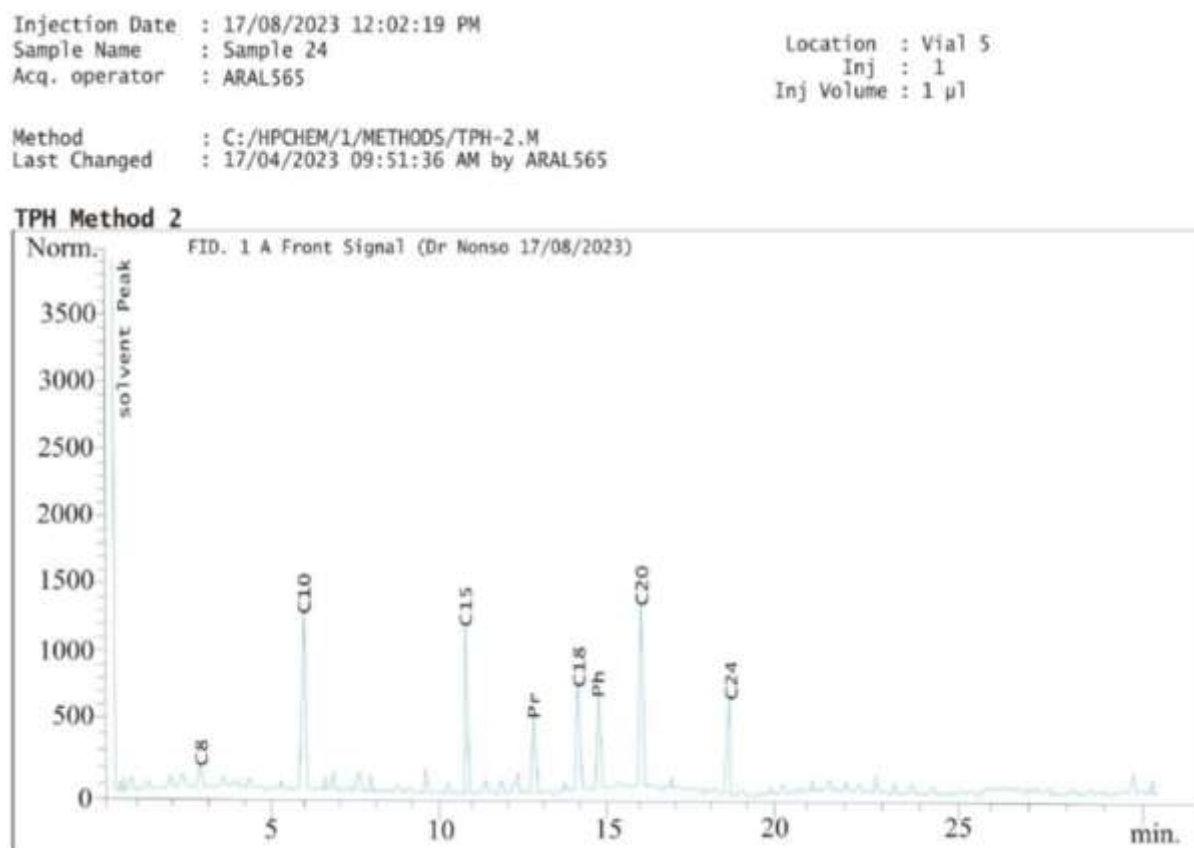
Figure 5: TPH Concentration after 4 weeks with 24ml E-Safe

```
Injection Date  : 17/08/2023 12:37:11 PM
Sample Name     : Sample 48                    Location  : Vial 6
Acq. operator   : ARAL565                           Inj  : 1
                                              Inj Volume : 1 µl

Method          : C:/HPCHEM/1/METHODS/TPH-2.M
Last Changed    : 17/04/2023 09:51:36 AM by ARAL565
```
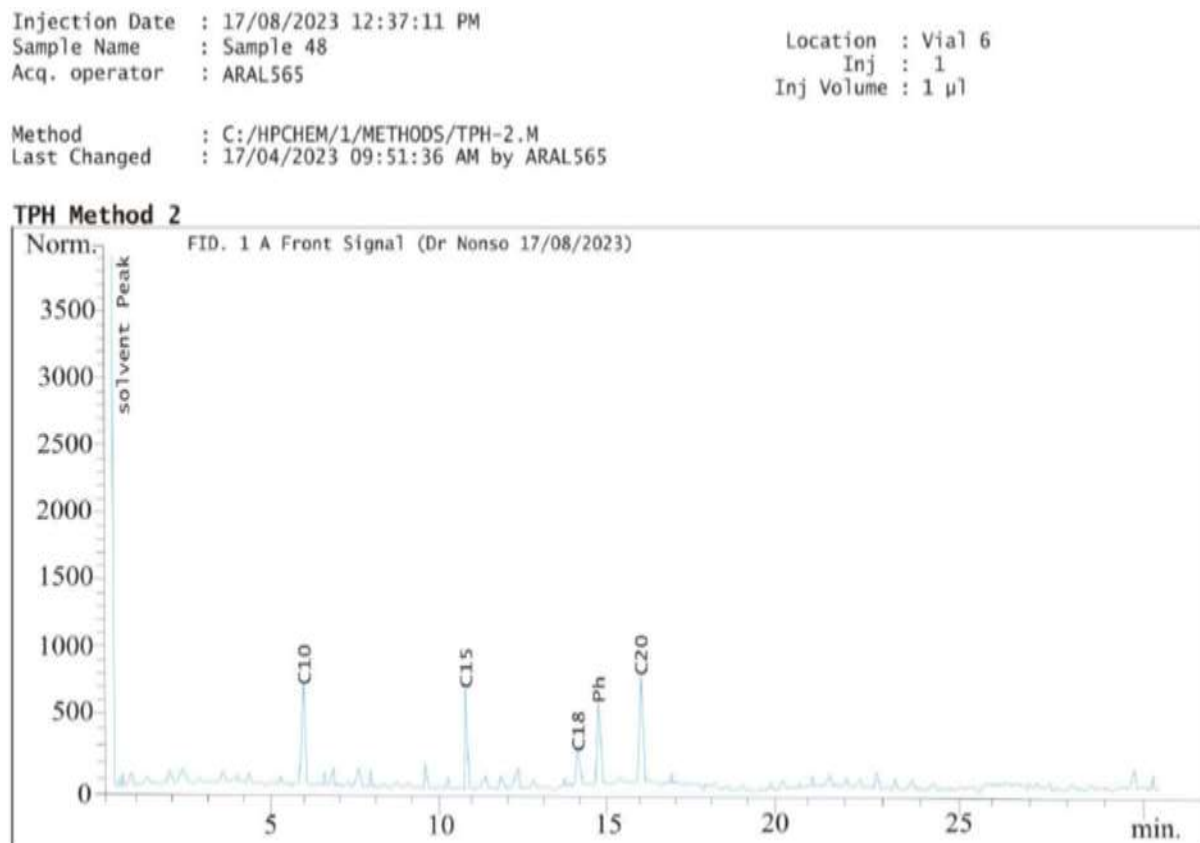

Figure 6: TPH Concentration after 4 weeks with 48ml E-Safe

CONCLUSION

This is a part of an ongoing research at our laboratory. The crux of this preliminary stage of the research was to investigate the effectiveness of E-Safe product in decontamination of hydrocarbon contaminated soil at various concentrations. Results obtained in this study shows that the product holds potential for crude oil polluted environments. Although the formulation of E-safe product is not publicly known, its mineral nutrients and possible hydrocarbon emulsifying activity could have acted as effective materials in the total removal of oil contaminants in the contaminated ecosystem. Research is still ongoing to ascertain the effects of the reclaimed contaminated soil on growth of crops in the tropics.

References

1. Iwegbue, C.M.A., Egobueze, F.E. and Opuene, K. (2006) Preliminary assessment of heavy metals levels of soil of an oil field in the Niger Delta, Nigeria. Int. J. Environ. Sci. Technol., 3(2), 167– 172.

2. Iwegbue, C.M.A., Nwajei, G.E. and Arimoro, F.O. (2007) Characteristic Levels of total petroleum hydrocarbons in soils, sediment and surface of an oil impacted area in the Niger Delta. Pak. J. Sci. Ind. Res., 50(4), 427– 250.

3. Ogundiran, M.B. and Osibanjo, O. (2009) Mobility and Speciation of heavy metals in soils impacted by hazardous waste. Chem. Speciat. Bioavail., 21(2), 59 – 69.

4. Reddy, K.R., Xu, C.Y. and Chinthamreddy, S. (2001) Assessment of electrokinetic removal of heavy metals from soils sequential extraction analysis. J. Harzard. Mater., B84, 279–296.

5. Reeuwijk. J.P. (1995) Procedure for soil analysis, Technical paper 5 ISRIC, Wageningen, The Netherlands.

6. Ryan, P.C., Wall, A.J., Hiller, S. and Clark, L. (2002) Insight into sequential chemical extraction procedure from quantitative XRD: A study of trace metals partitioning in sediment related to frog malformities. Chem. Geol., 184, 337– 357.

7. Salbu, B., Kreling, T. and Oughton, D.H. (1998) Characterization of radioactive particles in the environment. Analysts, 123, 843– 849.

8. Adepoju PA, Marcus AA (2000). Effect of processing on the quality and acceptabilityof two varieties of cowpea flour. In: Nkama, L., Jideani, J.A. and Ayo, J.A. (eds.).Proceedings of the 24th Annual Conference of NIFST held at Bauchi, Bauchi Statebetween 20th and 24th November, 2000. Pp 327 – 328.

9. Adoki A, Orugbani T (2007). Influence of nitrogenous fertilizer plants effluents ongrowth of selected farm crops in soils polluted with crude petroleum hydrocarbons.Afr. J. Agric. Res. 2(11):569-573.

10. Agbogidi, O.M. and Ejemete, O.R.(2005). An Assessment of the Effects of Crude OilPollution on Soil Properties, Germination and Growth of Gambaya albida (L.).Research Journal of Agricultural Science Technology 8(2): 148-155.

11. Benka-Coker, M.O. and Ekundayo, J.A. (1995). Effect of an Oil spill on SoilPhysicochemical Properties of a Spill Site in the Niger Delta Area of Nigeria. Journalof Environmental Monitoring and Assessment 36: 93-104.

12. Tao K, Liu X, Chen X, Hu X, Cao L, Yuan X. (2017). Biodegradation of crudeoil by a defined co-culture of indigenous bacterial consortium andexogenous Bacillus subtilis. Bioresour Technol. 224:327–32.

13. Nsikak U. Benson, Joseph P. Essien, Akan B. Williams, Godwin A.Ebong. (2019). Petroleum Hydrocarbons Accumulation Potential of Shellfishesfrom LittoralWaters of the Bight of Bonny, Niger Delta, Nigeria. Res J Environ Sci. 1(1):11–9.

14. Zauyah, S., Juliana, B., Noorhafizah, R., Fauziah, C.F. and Rosenani,A.B. (2004) Concentration and speciation of heavymetals in some cultivated and uncultivated ultisols and inceptisolsin Peninsular Malaysia. Paper presented in Supersoil2004; 3rd Australian

New Zealand Soils Conference, 5–9December, 2004 University of Sydney, Australia pp. 1 – 5.

15. Tessier, A., Campbell, P.G.C. and Bisson, M. (1979) Sequentialextraction procedure for speciation of particulate trace metals.Anal. Chem., 51, 844– 851.

16. Broughriet, A., Proix, N., Billon, G., Recourt, P. and Quddane, B.(2007) Environmental impacts of heavy metals dischargesfrom a swelter in Deule-cannal Sediments (Northern France):concentration levels and chemical fractionation. Water, Air SoilPollut., 180, 83 – 95.

17. Abollino, O., Aceto, M., Malandrino, M., Mentasticstic, E., Sarzanini,C. and Petralla F. (2002) Heavy metals in agriculturalsoils from Piedmont, Italy: Distribution, speciation and chemometricdata treatment. Chemosphere, 49, 545– 557.

18. Guerra AB, Oliveira JS, Silva-Portela RCB, Araujo W, Carlos AC, Vasconcelos ATR, (2018). Metagenome enrichment approach usedfor selection of oil-degrading bacteria consortia for drill cuttingresidue bioremediation. Environ Pollut. 235:869–80.

19. Curbelo FDS, Garnica AIC, Oliveira EL, Neto ELB. (2016). Oil removal in producedwater by adsorption using expanded and hydrophobized vermiculiteas adsorbent. Afinidad. 63(521):35–9.

20. Alvarez PJ, Vogel TM. (1991). Substrate interactions of benzene, toluene,and para-xylene during microbial degradation by pure culturesand mixed culture aquifer slurries. Appl Environ Microbiol.57(10):2981–5.