Biostimulation of Soil Artificially Polluted with Crude Oil Amendment with *Trichoderma* Specie

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Abstract

The contamination of soil with crude oil poses a great threat to the environment, affecting its fertility and overall ecosystem health. This project aims to investigate the potential of Trichoderma species as an amendment to stimulate the remediation of crude oil polluted soil. The effectiveness of Trichoderma species in enhancing the degradation of crude oil was evaluated by measuring key parameters such as total petroleum hydrocarbon (TPH) content, microbial activity. Preliminary results indicate that the addition of Trichoderma species significantly enhanced the degradation of crude oil in the polluted soil. The TPH content decreased in the treatment groups compared to the control group, from 2078 ppm to 312 ppm for T2 % and 3885 ppm to 1247 ppm for T10 %. PAHs have a reduction of 11.63 ppm to 0.11 ppm for T2 % while T10 % recorded 30.27 ppm to 2.83 %. Microbial activity, as indicated by the increased population of hydrocarbon-degrading bacteria, unamended set-up recorded 1.15 x 10^4 cfu/g – 4.8 x 10^5 while the amended recorded 2.3 x 10^5 cfu/g – 6.6 x 10^7 cfu/g. This showed a significant improvement in the Trichoderma-amended soil samples. These findings suggest that Trichoderma species have the potential to stimulate the bioremediation of crude oil contaminated soil. The metabolic capabilities of Trichoderma, such as hydrocarbon degradation and plant growth promotion, contribute to the overall improvement of soil health and restoration of ecosystem functionality. Further investigations are warranted to optimize the application of Trichoderma species in large-scale bioremediation projects.

Keywords: *Trichoderma spp. Stimulation, Crude Oil, Bioremediation, Nitrate*

1.0 Introduction

Soil pollution with crude oil is a major environmental problem that affects the quality of soil and water (Al-Shamary, *et al.*, 2023). Crude oil contains a complex mixture of hydrocarbons that are toxic to plants and microorganisms. The presence of crude oil in soil can lead to reduced soil fertility, decreased plant growth, and contamination of groundwater (Bello and Moses, 2020). The remediation of crude oil-contaminated soil is a challenging task that requires the use of effective and sustainable methods (Prasad, 2017).

Environmental degradation which results from oil spillage during extraction, processing, transportation and corrosion of pipeline or damage is one of many disasters that have been caused by humans. Oil contamination reduces the ability of soil to support the growth of plants, seeps into ground to contaminate ground water, and increase the presence of heavy metals

which can bioaccumulate and biomagnify causing adverse health effects (Varjani, et al., 2015; Varsha and Aapurna, 2016).

Petroleum hydrocarbons are majorly composed of alkenes and PAHs. Polycyclic Aromatic Hydrocarbons (PAH) are fused ring aromatic structures, very recalcitrant and have a high affinity for soil particles. Their accumulation in soil makes them more difficult to eliminate mainly because of their hydrophobic nature which gives them high persistence in the environment. Binding of PAH with other contaminants can prolong their persistence in environments as this association reduces the oxygen level needed by microbes for transformation while anaerobic transformation of PAH is limited (Deary *et al.,* 2022).

The use of microorganisms such as *Trichoderma* species has been proposed as a potential solution to remediate crude oil-contaminated soil. *Trichoderma* species are known for their ability to degrade hydrocarbons and improve soil properties (Cesare, 2015). *Trichoderma* species are also known to promote plant growth and enhance plant resistance to biotic and abiotic stresses (Chen and Zhuang, 2016).

It has been demonstrated that certain *Trichoderma* species enhance the pH, organic matter content, and nutrient availability of soil. A drop in pH caused by the presence of crude oil can impact a plant's ability to receive nutrients. Organic acids produced by *Trichoderma* species have the potential to decrease soil pH and increase nutrient availability (Lee, *et al*., 2022). Additionally, certain *Trichoderma* species are capable of producing organic matter-degrading enzymes, which enhance soil structure and water-holding ability (Marschner, 2021).

Trichoderma species' influence on soil parameters have been shown in a number of research. For instance, a study conducted in 2016 by Singh *et al.* Demonstrated that adding *Trichoderma harzanium* resulted in an increase in soil microbial biomass and enzyme activity. Shrestha, *et al*., (2020) study also demonstrated that Trichoderma support soil microbial biomass.

Trichoderma species are known for their ability to degrade hydrocarbons, including crude oil. The degradation of crude oil by *Trichoderma* species involves the production of enzymes such as lignin, laccase, and peroxidase. These enzymes can break down the complex hydrocarbons in crude oil into simpler compounds that can be used as a source of carbon and energy by microorganisms (Patowar*y et al*., 2017).

Several studies have reported the ability of *Trichoderma* species to degrade crude oil. For example, a study by Okerentugba and Ezeronye (2003) showed that *Trichoderma* viride was able to degrade 70% of crude oil in soil after 28 days of incubation. The application of *Trichoderma* viride led to an increase in soil microbial biomass and enzyme activity (Okerentugba and Ezeronye (2003); Chen and Zhuang, 2017).

Attempt to remediate hydrocarbon on polluted sites using physiochemical methods had been in existence since, but due to other implications their application was discouraged. An understanding of the impact of crude oil on indigenous microbial communities and identification of oil-degrading microbial groups as well as the *Trichoderma* specie contaminated soils are prerequisite for directing the management and cleanup of crude contaminated soil environment (Ogru and Olannye, 2021). The aim of the research was to

investigate the use of Trichoderma specie as a composing amendment to enhance the biostimulation of soil artificially polluted with crude oil. Its' objectives include (i) Enumeration and isolation of bacteria and fungal populations in crude oil contaminated soil (ii) assess the degradation efficiency of Trichoderma species on crude oil amended soil (iii) evaluate the changes in soil physicochemical properties after Trichoderma amendment.

Materials and Methods
Collection of Samples

The soil samples used were collected from the Agricultural farm land which was previously a dump site at Rumuosi Community, Rivers State where there is no previous history of oil pollution using hand soil auger at the topsoil between the depth of at 0 to 15cm, and 15 to 30cm at each point and mixed thoroughly to form a composite sample. The sample was kept in a clean perforated polythene bag and transported to the laboratory for analysis. The crude oil sample used was obtained from the same laboratory in Port Harcourt, Rivers State, Nigeria. The *Trichoderma* specie was isolated from the soil after series of processes.

Treatment of Soil Samples with Pollutant
The treatment of soil samples with pollutant was carried out as described by Mbagwu *et al.* (2021). In the laboratory, 500g of the soil sample was weighed and measured thrice, put in foil, autoclaved twice allowed to cool and put in sterile containers labelled T2 and T10.

Amendment treatments
Peptone water was prepared, put in a conical flask, autoclaved, cooled, and then the sub cultured *Trichoderma* specie was introduced and allowed to grow. 10ml and 50ml of crude oil were added to the sterile containers labeled T2 and T10 respectively. For T2%, 10ml of crude oil was added to the 2% of 500g soil sample, for T10%, 50ml of crude oil was added to the 10% of 500g soil sample and then 10ml of the sub cultured organism after growth in the peptone water was put in the T2% and T10% respectively. This experimental setup was allowed to stand for 60 days for which culturing and isolation of organisms was done on day 0, 30, and 60. The soil labeled UPS was used as the control for both the pollution treatment and amendment treatments. Each set was mixed thoroughly with a wooden spatula to obtain a homogenized mixture and enhance aeration.

Microbiological Analysis

Sample Preparation
200g of soil sample was measured and 20ml crude oil was also measured using a syringe and incorporated thoroughly in a container and left for 7 days. The ten-fold serial dilution was carried out after.
In this method, 1g of the soil was weighed and transferred aseptically into test tubes containing sterile 9ml diluents (normal saline) which gave an initial dilution of 1:10ml. Subsequent dilutions were carried out by transferring 1ml from the initial dilution to another test tube containing 9ml sterile diluents which gave rise to 1:100 dilution. This was repeated until a dilution of $1:10^{-6}$ was reached.

Isolation of Bacteria

Pure cultures of bacteria were obtained by aseptically streaking representative discrete colonies of different morphological types which appeared on the cultured plates (NA and HUB) onto freshly prepared pre-dried Nutrient agar plates and were later incubated at 37°C for 24hours. After pure cultures were obtained and preserved. The bacterial isolates were preserved frozen in 10% glycerol in bijou bottles for later use. The 10% glycerol was prepared by adding 90 ml of water into a 10 ml glycerol solution in a conical flask and thereafter 5 ml was dispensed into bijou bottles, which were sterilized and allowed to cool before the isolates were transferred using sterile wire loop.

Enumeration of Hydrocarbon Utilizing Bacterial (HUB)

The hydrocarbon utilizing bacteria (HUB) in the sample was determined using the standard plate count on Bushnell Hass medium. The vapour phase transfer method was adopted with slight modification as reported by Williams and Agunkwo, (2018). In this method, instead of adding 1ml of crude oil on sterile filter papers placed on the lid of Petri dishes, 1ml of crude oil (sterilized by heating) was placed on the Whatman filter paper. The hydrocarbonablastic organisms will utilize the vapour emerging from the filter paper. Ketoconazole antifungal agent was added into the Bushnell-Hass agar after sterilization to inhibit the growth of fungi. Aliquot from 10^{-2} dilution was transferred into the center of the agar and was evenly spread using a sterile bent glass rod. The inoculation was done in duplicates and incubated at 37^0C for 7days.

Enumeration of Hydrocarbon Utilizing Fungal (HUF)

Similar to the HUB, the HUF was enumerated on Bushnell Hass agar fortified with tetracycline antibiotics (to inhibit the growth of bacteria). Aliquot (0.1ml) was withdrawn from 10^{-2} dilution using a sterile pipette and plated out in duplicates on Bushnell-Hass agar plates. The plates were evenly spread with bent glass rod and were inverted in the lid of the petri dish containing sterile Whatman filter paper saturated with 1ml diesel oil (vapor phase transfer technique) in an inverted position and incubated at 30^0C for 7 days.

Characterization and Identification of Bacterial Isolates

The bacterial isolates were characterized by observing them microscopically and subjecting them to series of biochemical tests such as Gram stain, catalase, citrate, oxidase, coagulase, Methyl Red, Motility, indole, starch hydrolysis, Voges Proskauer and sugar fermentation tests. Further confirmation was done by comparing their characteristics with those of known taxa as outlined in Bergey's Manual of Systematic Bacteriology.

Enumeration of Total Heterotrophic Bacteria

The total heterotrophic bacterial counts of soil samples were determined using the standard plate count method on nutrient agar. A serial ten-fold dilution was prepared using 1g of soil and 0.1ml of 10^5 dilution was inoculated and plated in duplicates. Plates were properly labeled and incubated at 37^0C for 24 hours.

Enumeration of Fungi

The total heterotrophic fungal counts were enumerated using the standard plate count method on Potato dextrose agar (PDA) plates fortified with tetracycline antibiotics (to inhibit the growth of bacteria). In this method, aliquot (0.1ml) of 10^{-2} dilution were transferred on prepared

Potato Dextrose agar (PDA) plates. The plates were later spread evenly using sterile bent glass rod. Inoculation was done in duplicates and after inoculation, plates were incubated at 28^0C for 3-5 days. Enumeration of fungal counts was carried out after incubation, while distinct fungal colonies were morphologically characterized and sub-cultured on fresh PDA plates for further identification (Williams and Barisi, 2018).

Microscopic Identification of Fungi

To appreciate the microscopic feature of the fungi isolated, lactophenol cotton blue was dropped on a clean glass slide, little growth of the fungus was removed with a sterile inoculating needle, and the preparation was covered with a clean cover slip and examined under the microscope with x10 magnification. Microscopic examination and morphological characteristics were noted and compared with existing authorities.

Physicochemical Analysis

The physicochemical parameters of polluted samples were analyzed; that of set up T2% and T10% were analyzed on days 0 and 60. The parameters that were analyzed include; Total petroleum hydrocarbon content (TPH), Polyaromatic hydrocarbon content (PAH), iron, lead, nickel, vanadium aluminum, electric conductivity (EC), pH, nitrate, and phosphate.

Determination of pH

The pH of soil sample was determined by the American Public Health Association (APHA) Standard Method. The pH meter was switched on and allowed for some time. It was then calibrated with buffer solutions of high pH range between 8 and 9 as well as a lower pH range between 1 and 6 by dipping the electrode into the buffer solutions. Soil sample(10g) was weighed into 100ml beaker; 50ml of distilled water was then added to allow immersion of the electrode, mixing was carried out by stirring frequently for few minutes. Then beaker was allowed to stand for 15 minutes. The electrode was immersed into the sample. The pH value for each sample was recorded accordingly.

Determination of Total Nitrogen

Adopting American Public Health Association (APHA) Standard Methods (APHA, 2012. A total of 1.0g of oven dried soil sample was weighed into a labeled dry and clean digestion tube. Then 15 ml digestion mixture was added to each tube. This was digested at 360^0C for 2 hours. The solution should now be colorless and remaining sand while if solution in still colored, heat for a further 1 hour, allow cooling. Add about 25ml distilled water and mix well until no soil dissolved. Allow cooling, make up to 50ml with distilled water and mix well. Allow settling so that a clear solution can be taken from the top of the tube for analysis.

Determination of Phosphate (PO$_4^{3-}$)

The phosphate levels for the samples were determined using an ultraviolet (UV) spectrophotometer. 25ml of 2.5% Acetic acid was added to 1g of soil sample and shaken for 30minutes. The suspension was filtered through a filter paper. 10ml of the extract was transferred into 50ml volumetric flask. Extract was diluted with distilled water until the flask is about 2/3 full. 2ml of Ammonium Molybdate reagent was added and mixed with extract. 2ml of stannous chloride was also added and mixed; the solution was diluted to 50ml mark with distilled water. The flask was allowed to stand for 30minutes, and the absorbance was measured at wavelength of 690nm.

Determination of total petroleum hydrocarbon and polyaromatic hydrocarbon

Soil sample of 5g was weighed into the brown extraction container and concentrated sodium sulphate (Na_2SO_4) was added to remove its water molecule. The concentrated Di-chloromethane (DCM) was added to extract the hydrocarbon content that was in the soil sample. Also, the solution was filtered through the funnel that was packed with cotton wool, Na_2SO_4 and silica gel. Then the filtrate was passed through the silica column and collected in the beaker and then blown down the nitrogen gas until it is finally evaporated. Before the extracted sample was ran in the gas chromatography, the content in the evaporated beaker was mixed with 1ml of DCM after which 1 microliter (0.001ml) was injected into the GC system with a syringe. Furthermore, the heavy metals were determined using atomic adsorption spectrophotometer.

Statistical Analysis

The results were expressed as mean ± standard deviation of two replicates. Analyses of variance (ANOVA) were carried out using SPSS (version 25.0) to check for significant difference and mean values were separated using the Ducans multiple range test (DMRT) at $P \leq 0.05$. The percentage occurrence was calculated on Microsoft Excel (version 2021).

Results

Table 1: Morphological Characteristis and Probable Organism of the Isolates

(0-30)	MORPHOLOGY	PROBABLE ORGANISM
HUF A (0-15)	Slow, green, segmented, circular, dry, , mycellia	*Penicillium sp.*
B (0-15)	Green, mycellia, fast growing	*Trichoderma sp.*
PDA A (0-15)	Green, mycellia, fast growing	*Trichoderma sp.*
C (0-15)	Slow, green, segmented, circular, dry, , mycellia	*Penicillium sp.*
PDA A (15-30)	Green, segmented, drowny, rough, dry, mycelia, circular, flat surface, white margin, 2.3cm	*Trichoderma sp.*
B (15-30)	Greenish, dry, mycellia, white margin	*Trichoderma sp.*
C (15-30)	Green, mycellia, fast growing	*Trichoderma sp.*
D (15-30)	Black, mycellia, fast growing, conidiosphores	*Aspergillus niger*
E (15-30)	pink to yellow, cottony, mycellia, conidia	*Fusarium sp.*

Table 2: Hydrocarbon utilizing bacteria Count for samples

Samples	HUB (Cfu/g) Unamended	HUB (Cfu/g) Amended

|		
A	1.15×10^4	2.3×10^5
B	3.0×10^4	3.0×10^6
C	1.25×10^5	2.65×10^7
D	2.2×10^5	1.50×10^7
E	4.82×10^5	6.6×10^7
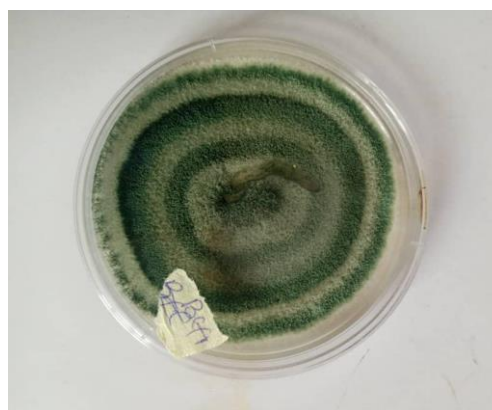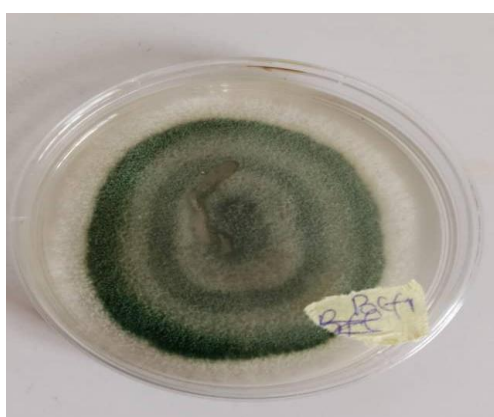
Plate 1& 2: Day 4 & 5 Of *Trichoderma* Growth (7.8Mm & 8mm) Respectively

Table 3: Physicochemical Parameters of Treatment Set-ups

| S/N	PARAMETERS	T2% Day 0	T2% Day 60	T10% Day 0	T10% Day 60
1	pH	6.74	5.83	6.91	5.96
2	EC, µS/cm	247.80	217.88	217.20	223.52
3	Nitrate, mg/kg	7.412	5.793	5.938	4.054
4	Phosphate, mg/kg	0.844	0.765	0.297	1.150
5	Fe, mg/kg	17.115	28.095	13.673	23.821
6	Pb, mg/kg	0.145	2.396	<0.001	3.417
7	Ni, mg/kg	0.212	4.793	0.136	4.420
8	V, mg/kg	<0.001	< 0.001	0.013	0.053
9	Al, mg/kg	<0.001	< 0.001	< 0.001	< 0.001
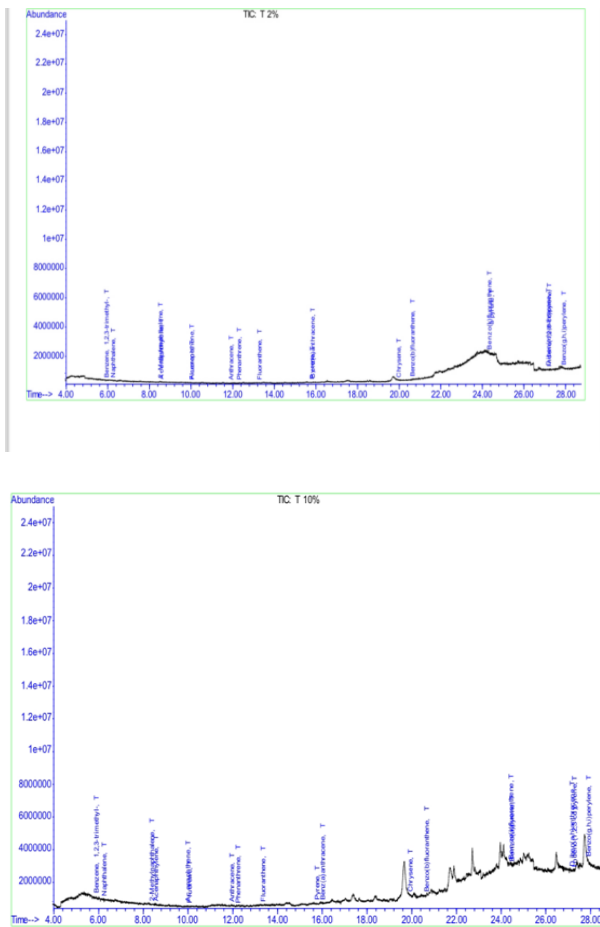
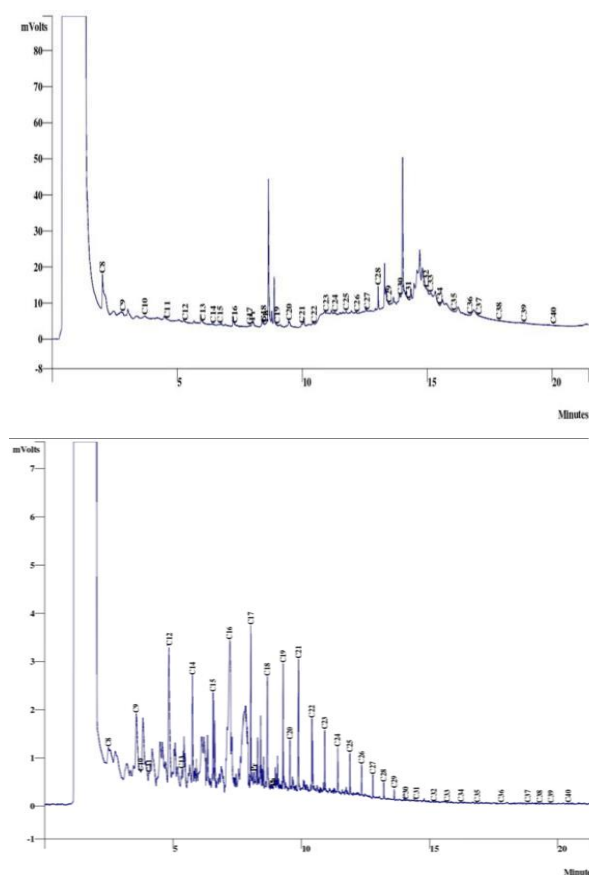**FIG. 1: Chromatogram profile showing PAH of sample T2% and T10% at day 0**
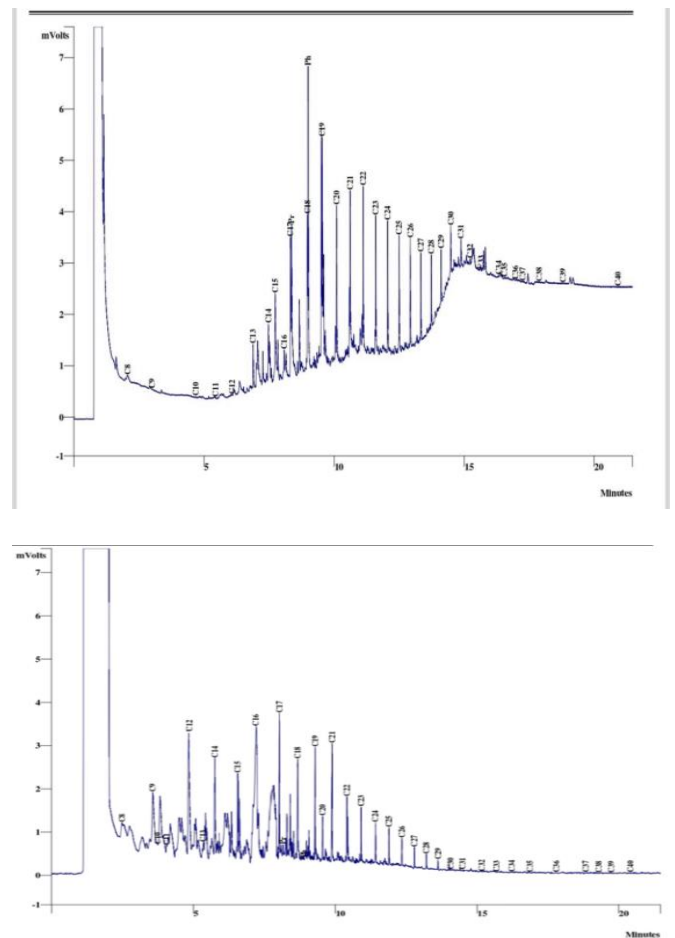
FIG. 2: Chromatogram profile showing TPH of sample T2% and T10%at day
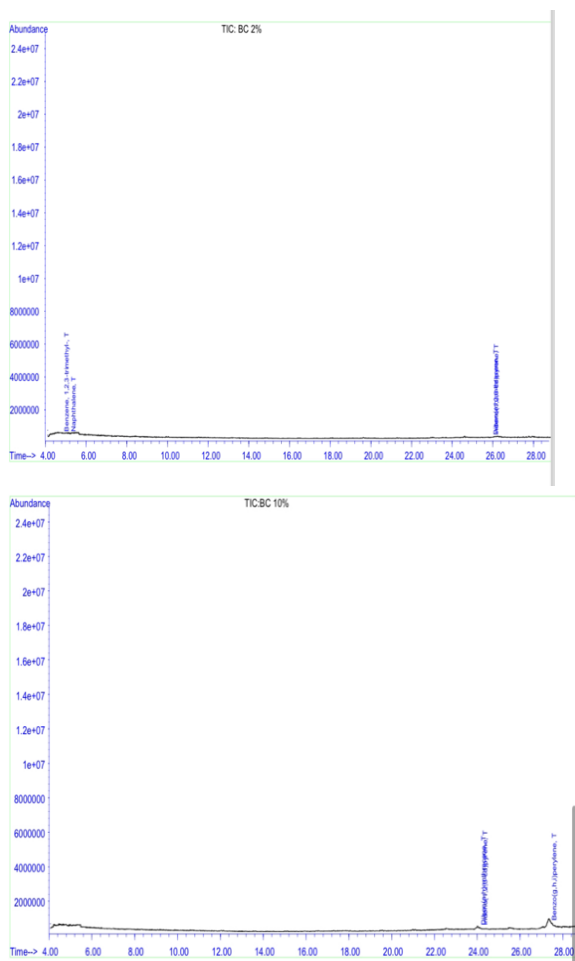
FIG. 3: Chromatogram profile showing PAH of sample T2% and T10% at day 60

FIG. 4: Chromatogram profile showing TPH of sample T10% at day 60

Discussion

The potential of *Trichoderma* species as a sustainable and cost-effective solution for remediating crude oil-contaminated soil. The ability of these fungi to degrade petroleum hydrocarbons and stimulate microbial activity in soil is promising for future applications in environmental remediation (Boopathy *et al.*, 2020).

The bacterial and fungal types identified in the control for the first week were higher than those recorded for the contaminated soils without amendment (table 1 and 2). The high bacterial and fungal types observed in amended soils could be ascribed to the presence of *Trichoderma* sp. which could harbour microorganisms. More so, the bacterial and fungal isolates in this study have been reported in previous studies. Mbagwu *et al.*, (2021) isolated members of various genera such as *Bacillus, Lysinibacillus,* and *Enterobacter* from crude oil polluted soil. Yahemba *et al.*, (2022) isolated *Bacillus* sp, *Pseudomonas* sp, *Acinetobacter* sp, *Micrococcus*

sp and *Staphylococcus* which agreed with the present study except the presence of *Staphylococcus* sp as Hydrocarbon utilizing bacterial which does not concur with the present study. The percentage occurrence of hydrocarbon utilizing bacterial in the present study is in agreement with theirs which showed that *Bacillus* sp was the prevalent isolate followed by *Pseudomonas* sp. and *Alcaligenes* sp. were observed to have the highest numbers of growth on the contaminated soils. This result is in line with Ugoh and Moneke (2011) who reported that the bacterial isolates from the soil contaminated with petroleum products from the sites showed *Pseudomonas sp, Bacillus sp* and *Klebsiella spp.* having highest percentage occurrence frequency of 60%. Similarly, in weeks 3 and 4, *Bacillus sp.* and *Pseudomonas sp* were observed to have the highest occurrence of growth on the crude contaminated soils. The prevalence of *Bacillus* sp as crude oil utilizing bacteria could be due its ability to survive harsh condition by the formation of spores. Its hydrocarbon degrading enzyme system and ability to emulsify petroleum hydrocarbon is another reason for its high occurrence in Nigerian soils(Yahemba *et al.*, 2022). *Pseudomonas, Bacillus* and *Micrococcus* isolated in this study corresponds with the work of Idowu and Ijah (2017).

The prevalence of *Aspergillus* sp in the diesel contaminated soil could be attributed to its ability to utilize the crude oil. This agreed with Mbagwu *et al.,* (2021), who reported similar findings. In our research four fungi genera were identified and *Trichoderma* sp was the most prevalence (table 1). The fungal isolates in this study have been reported in a previous study to be associated with hydrocarbon utilization (Segun, et al., 2017). In a previous study of soil samples contaminated with crude oil, fourteen fungal genera belonging to *Alternaria sp., Aspergillus sp., Cephalosporium sp., Cladosporium sp., Fusarium sp., Geotrichum sp., Mucor sp., Penicillium sp., Rhizopus sp. Trichoderma sp., Candida sp., Rhodotolura sp., Saccharomyces sp.* and *Torulopsis* sp from the control soil, with five hydrocarbon utilizing fungi identified out of the fourteen. In this study, *Cephalosporium* sp was not isolated. The hydrocarbon utilizing bacteria and fungi identified in this study have been shown to have the ability to utilize crude oil as carbon source (Idowu and Ijah, 2017; Obukohwo et al., 2020).

There was a high fluctuation in the physicochemical parameters of the soil during the study period. Nitrogen, phosphate, organic carbon, and soil organic matter increased throughout the study in amended samples. Unamended samples showed decrease. The increase in these nutrients could be as a result of the addition of *Trichoderma sp.* which increased the nutrients in the amended samples. Nitrogenous compounds and other necessary nutrients present were reasons for this increase. This result agreed with Yahemba *et al*., (2022) who observed similar trend but differs with that of Nwogu *et al*., (2015), who observed decrease in nitrogen, phosphorus and potassium levels in amended samples for a period of 42 days. The ratio of organic waste to soil used by Nwogu *et al.* (2015) could have been the reason for the decrease in nutrient content. This study also showed that the pH of all the amended samples increased with time. This is similar to the report of Adams *et al.,* (2015) who also observed increase in pH with time after amending soil with organic manure. Bacterial growth and activity are readily affected by pH and in this study, The pH ranged from 6.2 to 7.8. This observation slightly differs from the pH range (6.0 to 8.9) as the best pH range for bioremediation of hydrocarbon polluted soils and that these changes in pH level could be due to the release of acidic and alkaline intermediates and final products during biodegradation of hydrocarbons, which has an effect on the pH.

The use of *Trichoderma* species for the remediation of crude oil-contaminated soil has several challenges. One of the major challenges is the variability in the effectiveness of *Trichoderma* species in different soil types and environmental conditions. The effectiveness of *Trichoderma* species can also be affected by the presence of other microorganisms in soil, which can compete for nutrients and space (Chen and Zhuang, 2016).

Another challenge is the cost-effectiveness of using *Trichoderma* species for the remediation of crude oil-contaminated soil. The production and application of *Trichoderma* species can be expensive, especially in large-scale applications. The use of *Trichoderma* species for the remediation of crude oil-contaminated soil also requires careful monitoring to ensure that the remediation process is effective and sustainable (Xuezhi, 2020).

Despite these challenges, the use of *Trichoderma* species for the remediation of crude oil-contaminated soil has several future prospects. The development of new strains of *Trichoderma* species that are more effective in degrading crude oil and promoting plant growth can improve the effectiveness of the remediation process. The use of bioreactors and other innovative technologies can also improve the cost-effectiveness of using *Trichoderma* species for the remediation of crude oil-contaminated soil (Xuezhi, 2020).

However, further research is necessary to optimize the concentration and application methods of *Trichoderma* species to achieve maximum efficiency in soil remediation. Generally, the total heterotrophic bacteria and fungi in the crude oil contaminated soil with *Trichoderma* sp. had higher bacterial and fungal load compared to soil contaminated with crude oil without *Trichoderma sp.* amendment. The reason for this increase in microbial count of the amended soil might be as a result of the addition of *Trichoderma sp* which could contain detectable quantities of nitrogen and phosphorus which are essential nutrients for microbial growth (Mercl *et al.*, 2020).

More so, the high crude utilizing bacteria and fungi observed in crude contaminated soil amended with *Trichoderma sp* could also be ascribed to the effect of the *Trichoderma sp* in enhancing growth of the hydrocarbon degraders as shown in table 2 (Ogbonna *et al.*, 2020).

Trichoderma sp is known to be an organic stimulant that helps in the stimulation of hydrocarbon in the soil. The addition of this stimulant as nitrogen source helps to increase the microbial population of a contaminated site and also enhance the degradation of the crude oil in the soil environment (Eslahi, *et al*., 2021; Okafor *et al*., 2016).

According to Labud *et al*., (2017), crude oil causes reduction in species richness, evenness and diversity when spilled in a soil environment. Lower hydrocarbon-utilizing bacteria counts in the unamended sample could be as a result of depletion of limiting nutrients. Furthermore, Hawrot-Paw *et al.*, (2020) opined in their study that fuels introduced into the soil could stimulate or reduce the number of bacteria depending on the type of contamination and its dose. It was observed that crude contaminated soil with and without *Trichoderma sp* had higher fungal count than the unpolluted soil. This observation could be ascribed to the ability of fungi to utilize crude oil products as sources of carbon and energy. In the study carried out by Hazim and Al-Ani (2019), soil contamination with 5% and 10% crude oil increased fungal counts by 73% and 139%, respectively. Their study demonstrated the capacity of fungi to use diesel

hydrocarbons as an energy source and their potential ability to biodegrade crude oil (Hawrot-Paw *et al*., 2020). This is similar to our result, Total petroleum hydrocarbon for T2% recorded 99.05 % reduction while T10 % recorded 90.65 %. For PAHs, there was 84.98 % reduction for T2 % while T10 % recorded 67.90 % values can be seen in figures (1, 2, 3 and 4).

The Biodegradation recorded in the unamended soil sample could be due to non-biological factors such as evaporation, photo-degradation (Williams and Amaechi, 2017); volatilization, adsorption, abiotic factors (temperature and pH). Reduction of petroleum hydrocarbon in unamended sample has also been reported by previous study (Nwogu *et al.,* 2015; Idowu and Ijah, 2017).

CONCLUSION

Studies have shown that *Trichoderma* species could significantly reduce the TPH content and enhance microbial activity in contaminated soil. This research contributes to the development of sustainable and environmentally friendly approaches to address crude oil pollution in soil. Further studies are needed to explore the full potential and application of *Trichoderma* species in large-scale soil remediation projects.

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