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Bioremediation Potential of Bioaugmented Fungi-Bacterial Effective-Microorganisms in Crude Oil - Contaminated Soil Microbial Properties

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Abstract
Research experiment was conducted at Rivers State University Teaching and Research Farm, Faculty of Agriculture on potential of Bio-augmented fungi-bacterial effective-microorganisms for bioremediation of crude oil contaminated soils. Two factor experiments consisting of five levels of bio-augmented fungi-bacterial effective-microorganisms (BFBE) and a level of Bonny light crude oil were replicated five times and fitted into a Completely Randomized Design (CRD). The treatments were incubated and monitored monthly for three months and samples collected for Laboratory analysis of soil chemical and physical properties. The results from the Laboratory were statistically analyzed using analysis of variance (ANOVA) in a Minitab software and the means separated using a Standard Error at p<0.05. Results showed that BFBE applications increases soil microbial populations such as bacterial count, hydrocarbon utilizing bacterial, fungi population, hydrocarbon utilizing fungi. Bacterial isolated in BFBE treated soil are Bacillus sp, Corrynebacterium, Enterobacter, Staphylococcus, Rhodococcus rhodochrous, Cyanobacteria, Escherichia, Pseudomonas sp., Spirillum sp., Serratia sp., Streptomyces sp. Whereas, fungi such as Aspergillus niger , mucus, Aspergillus fumigates, Fusarum, and chlamydosporum which are found to utilize hydrocarbon bioaugmented fungi-bacterial effective-microorganisms should be used at 100ml to 200ml per liter of water to enhance and improve soil microbial properties.

***Keywords**: Bioremediation, Potential, Bioaugmentation, Effective-microorganisms, Microbial*

INTRODUCTION

Bioremediation is a process whereby the use of microorganisms is mainly to detoxify or degrade the level of contaminants in the soil (Chandran, *et al.,* 2020). The microorganisms break down contaminants by using them as energy source or co- metabolizing them with energy sources. These effective microorganisms help in the breakdown of toxic chemicals or render them less hazardous, it helps in making soil nutrient available for plants, improves the structure of the soil, increases its fertility, fixes the nitrogen in the soil and enhances nutrient uptake, radically improves biological diversity, it also accelerates the decomposition of organic matter, residue, compost manure, etc (Kleiber, *et al.,* 2014).

Soil contaminated by petroleum hydrocarbon can affect soil health and can harm soil microorganisms reducing their numbers and activities. Crude oil is one of the commonest petroleum hydrocarbons in the soil.

Oil pollution is difficult to treat. Petroleum hydrocarbon-degrading bacteria have evolved as a result of the existing proximity of naturally occurring petroleum hydrocarbon in the soil. Such organisms (bacteria) are a candidate for the treatment of oil contamination (Ron and Rosenberg 2014, Smith, *et al.,* 2015). Therefore, EM has been screened and utilized to degrade contaminated products in agricultural soil. Effective microorganisms (EM) are a consortium of beneficial microorganisms; these microorganisms change the balance from degeneration to regeneration (EPA 2010). EM method is non-toxic and relatively cheap. It affects the availability of available nutrients (Kleiber, *et al.,* 2014). The contaminated soil has a high negative effect to soil microorganisms by reducing their numbers and activities and these soil microbes are what help make nutrients available to plants whereas the EM works by being dominant over other soil microbes in the contaminated soil, as a result, encouraging a bulk of microbes in the soil to suppress or degrade the oil spilled content in the soil. Plants would therefore grow exceptionally well in soil inhabited, dominated by EM which has bio remediated the content of contamination in the soil (Sun, *et al.,* 2014). The use of EM to deal with crude oil contamination has become a promising technology because of its low cost and eco-friendly nature (Guerra, *et al.,* 2018). Presently, hydrocarbons indicate one of the most significant sources of environmental impurity affecting the soils for farming operations.
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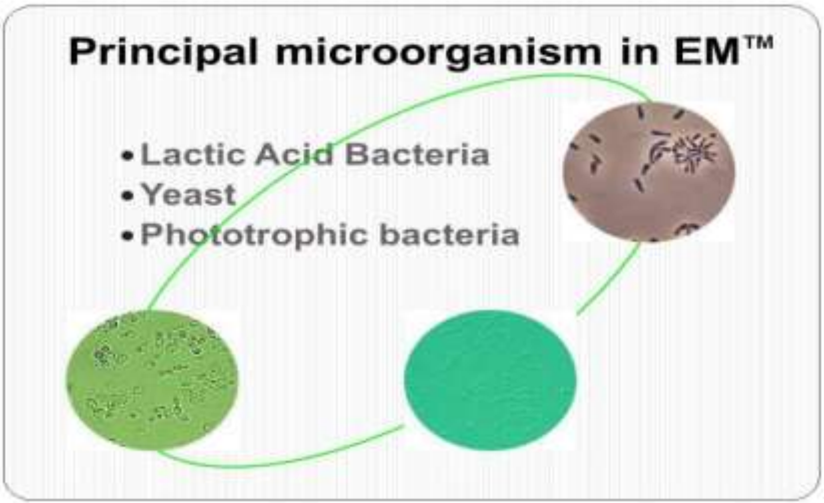
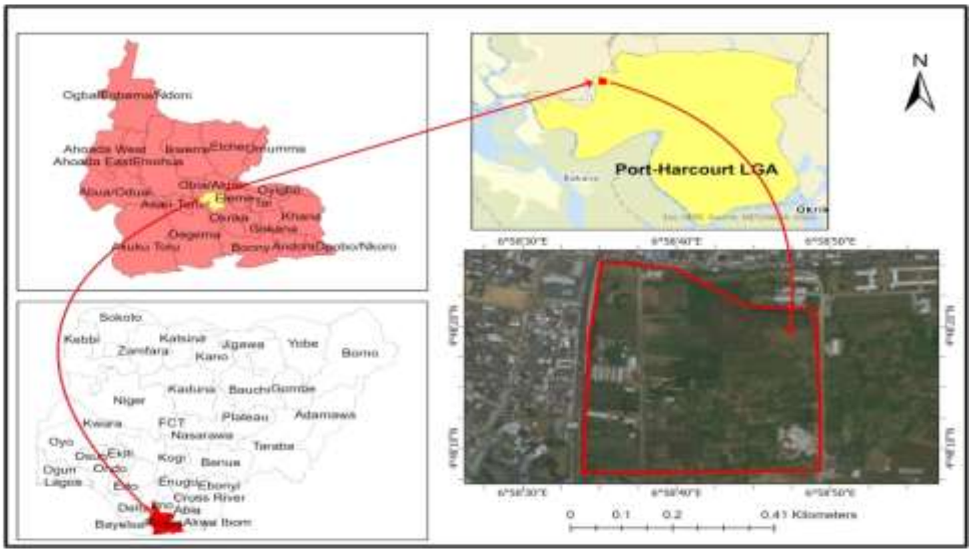


Figure. 2.1: Source: Integrated Bio-Product LLC (2020)

The perception of effective microorganisms (EM) is centered on the inoculation of the substrates to shift the bacterial stability and thus create an enhanced ecology that favors better productivity. (Ene and Opurum., 2023). Photosynthetic bacteria are the backbone of the EM, working synergistically with other microorganisms to provide the nutritional requirement to the plant and also reduce the disease problem. There are primarily 5 types of microbes used to formulate EM solution. Photosynthetic microbes (Phototrophic bacteria): are independent self-supporting microorganisms. These bacteria synthesize amino acids, nucleic acids, bioactive substances and sugars, substances from secretions of roots, organic matter (carbon) by using sunlight and the heat of soil as sources of energy. Lactic acid bacteria: produces lactic acid from sugars. Food and drinks such as yogurt and pickles have been made by using lactic acid bacteria. However, lactic acid is a strong sterilizer. It suppresses harmful microorganisms and increases the rapid decomposition of organic matter. Yeasts: synthesize antimicrobial and useful substances for plant growth from amino acids and sugars secreted by photosynthetic bacteria, organic matter, and plant roots. *Actinomycetes*: are the structure of which is intermediate to that of bacteria and fungi, produces antimicrobial substances from amino acids secreted by photosynthetic bacteria and organic matter. These antimicrobial substances suppress harmful fungi and bacteria. Fermenting Fungi: such as *Aspergillus* and *Penicillium* decompose organic matter rapidly to produce alcohol, esters, and antimicrobial (Ene and Opurum., 2023). The objectives of the study is to determine the effect of fungi-bacteria effective- microoganisms on soil total heterotrophic bacterial (THB) and total fungi count (TFC), evaluate the hydrocarbon utilizng bacterial (HUB) and hydrocarbon utilizing fungi, identify bacterial and fungi in hydrocarbon polluted soil, and determine the optimum level of Effective microorganism for bioremediation of crude oil contaminated soils.

MATERIALS AND METHODS

This experiment was conducted in a screen house located at the Teaching and Research Farm and the Soil Science Laboratory of Rivers State University (RSU) located at Port Harcourt. It lies between (04° 48' 15.6" N, and 006° 58' 40.9" E) and Elevation of 13 meters above sea level. Port Harcourt is in the humid forest zone which has an average elevation of ten meters above sea level. The mean annual rainfall is 2400mm, usually in a monomodal distribution lasting from March to November. There is usually a dry period between December and March with little or no rain, the wettest months are between July and October (Ikpe *et al.* 2003). Temperature varies from 27^{O}C from February to April (warmest months) to 25^{O}C in July and August (the coolest months). Relative humidity remains high throughout the year and varies from 78% in February (the driest month) to 89% in July and September (rainy months) (Ene e*t al.* 2018).



Map of the Study Area

Surface soil samples (0-15cm depth) were collected from the Teaching and Research Farm in Rivers State University (RSU) using a shovel. 10kg of the soil was weighed and transferred into sampling buckets of known volume (6,005.25cm^3). The soil samples were collected before pollution and after incubation. Bonny light Crude oil was obtained from Nigerian National Petroleum Corporation (NNPC) Port Harcourt.

The research consists of two treatments, bio augmented fungi-bacterial effective-microorganisms and crude oil. Six levels of bio augmented fungi-bacterial effective - microorganisms (0ml, 50ml; 100ml, 150ml, 200ml and crude oil only). Each treatment was replicated 5 times. One litre of water containing the different volumes of bio augmented fungi-bacterial effective -microorganism were applied into the contaminated soil to field capacity and allowed to incubate for six (6) days to allow penetration of the crude oil and monitored for one month. The two factor experiments were fitted into a Completely Randomized Design (CRD).

LABORATORY ANALYSIS

Enumeration of Bacteria

Soil sample were collected and put into a sterile specimen bottle, the samples were immediately taken to a laboratory for microbial analysis (Adoki and Orugbani, 2007). Total number of microorganisms in untreated soil and treated soil were determined, and the planting dilution technique using nutrient agar medium. The soil sample were processed using 1g soil sample suspended in a conical flask containing 9ml of sterilized distilled water and thoroughly shaken by 15 minutes. About 1ml of sample was serially diluted with sterilized distilled water up to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 0.1ml of appropriate dilution was spread plated onto the surface of sterile nutrient agar medium (for heterotrophic bacterial) inoculated plate were incubated at 25-30^{0}c for 24 hours and colony forming unit counted and calculated.

Total number of colonies gram of soil = $\dfrac{Number\ of\ colonies\ (CFUS)}{Dilution\ Factor\ X\ \ Amount\ plated}$

CFU = Colony Forming Unit

Enumeration of Fungi

One gram of top soil sample was collected and put into a sterile specimen bottle. The samples were immediately taken to the laboratory for fungi analysis (Adoki and Orugbani., 2007). The total number of microorganisms in untreated soil and treated soil were determined, and the planting dilution technique using potato dextrose agar medium. The soil samples were processed using soil sample suspended in a conical flask containing 9ml of sterilized distilled water and thoroughly shaken for 15 minutes. 1ml of sample was serially diluted with sterilized distilled water up to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 0.1ml of appropriate dilution was spread plated onto the surface of sterile potato dextrose agar medium (for heterotrophic fungi). Inoculated plats were incubated at 25-30^{0}C for 48 hours and colony forming units were counted and calculated.

Total number of colonies/gram of soil = $\dfrac{Number\ of\ colonies\ (CFUS)}{Dilution\ factor\ x\ Amount\ Plated}$

CFU = colony forming Unitc

Hydrocarbon Degrading Bacteria and Fungi

For hydrocarbon degrading bacteria and fungi, 1ml of crude oil was added into the media before dispensing into Petri dish and incubated for 24 to 48 hours respectively.

Isolation, Purification and Characterization of Bacteria

Serial dilution of soil samples was done, up to 10^{-3} dilution. Approipraite dilutions were plated onto nutrient agar medium and other agar media depending on the physiological bacterial type. The plates were incubated at 37^0C for 24- 48hrs. The pure cultures were obtained based on the colony characteristics and the pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the bacterial isolates. Individual bacteria were isolated by spread –plating on the solid medium. 0.1ml portion of each sample was pipetted and plated out on the solid medium. A ware-loop was sterilized with alcohol and flame was used to spread the inoculums evenly on the plates. The plates were incubated at 37^0C for 24-48hrs. Purity of isolates was achieved by sub-culturing discrete colonies to freshly prepared sterile nutrient agar plates by dissolving 2.9g of nutrient agar in 100ml of distilled water and autoclaved at 121^0C for 15 min. Nutrient agar was thoroughly shaken and then poured into washed bijou bottles which were tightly closed. The bottles were sterilized in the autoclave at 121^0C for 15min. After autoclaving they were slanted and allowed to solidify. Slant culture medium was inoculated with the purified bacterial culture obtained by the isolation and purification processes. Characterization of isolates was done by observing morphological and colonial characteristics of the isolates on the nutrient agar plates. The morphological tests were: Gram reaction, cell shape and size, endospores, position of endospores, motility, while the biochemical tests were Catalase, Oxidase, Urease and Coagulase tests, for the identification of isolates.

RESULTS

The results of effect of bioaugmented fungi-bacterial effective-microorganisms (BFBE) on total heterotrophic bacterial count in a crude oil contaminated soils were presented in Fig.1. The results showed that, 200ml BFBE recorded a higher count (1.2×10^7cfu/g) of soil while 50ml of BFBE records the least population of bacterial count (2.4×10^7cfu/g). Crude oil contaminated soil recorded 5.7x107cfu/g. The bacterial population increased with increased level of concentration of BFBE, the initial bacterial count of the soil samples used during the research recorded a bacterial population of (1.3×10^6cfu/g) in the first month after application of the treatments. Fungi count in a crude oil contaminated soils in first month of bioremediation is presented in Fig. 4.2. The results showed that, 200ml BFBE recorded a higher count (8.9×10^4cfu/g) of soil, followed by 150ml BFBE (4.5×10^4cfu/g) while crude oil contaminated soil only recorded the least fungi population (1.0×10^4cfu/g). Fungi population increased with increased level of concentration of BFBE. Hydrocarbon utilizing bacteria count in a crude oil contaminated soils is presented in Fig. 4.3. The results showed that, 200ml BFBE recorded a higher count (4.4×10^7cfu/g) of soil, followed by 150ml BFBE (3.7×10^7cfu/g), 100ml BFBE (3.4×10^7cfu/g), while crude oil contaminated soil only recorded the least hydrocarbon utilizing bacteria population (7.0×10^7cfu/g). Hydrocarbon utilizing bacteria population as observed in Fig.4.3. increased with increased level of concentration of BFBE, the initial hydrocarbon utilizing bacteria count of the soil samples used during the research recorded a population of (4.0×10^7cfu/g) in the first month after application of the treatments. Hydrocarbon utilizing fungi count in a crude oil contaminated soils in the first month after contamination is presented in Fig.4.4. The results showed that, 200ml BFBE recorded a higher count (3.3×10^4cfu/g) of soil, followed by 150ml BFBE (2.0×10^4cfu/g), 100ml BFBE. 50ml, crude oil only, and the initial soil recorded the same utilizing fungi count ($1{,}0\times10^4$cfu/g).

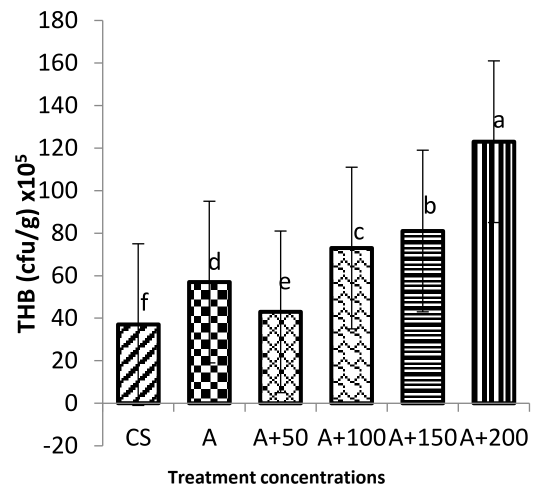


Fig..1. THB count in BFBE Treated Soil

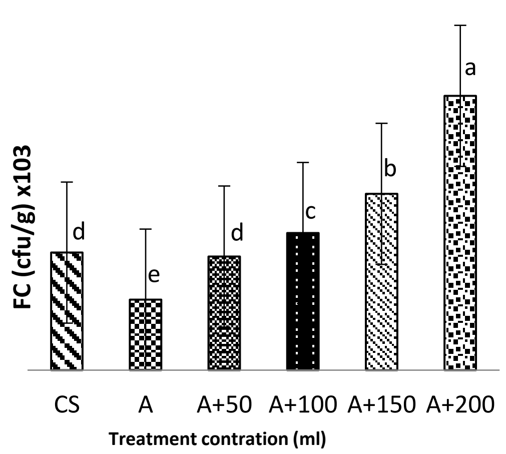


Fig..2. FC count in BFBE Treated Soil

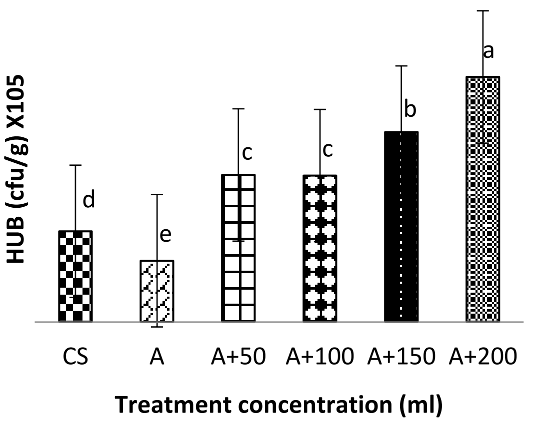


Fig..3. HUB count in BFBE Treated Soil

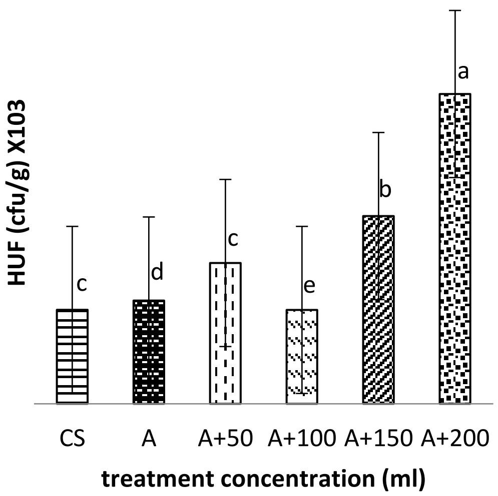


Fig.4. HUF count in BFBE Treated Soil

KEY: A = crude oil contaminated soil, BFBE = Bioaugmented fungi-bacteria effective-microorganisms, A+50 = crude oil contaminated soil + 50ml of BFBE, A+100 = crude oil contaminated soil + 100ml, A+150 = crude oil contaminated soil + 150ml of BFBE, A + 200 = crude oil contaminated soil + 200ml of BFBE, THB = Total heterotrophic bacterial

Table 1: Distributions of Bacterial Isolates in Bio-augmented fungi-bacterial effective-microorganisms Treated Soils

Treatments	Distribution of isolate in soil Bacterial	Distribution of isolate in soil Fungi
CS	*Streptomyces sp., Corrynebacterium, Enterobacter*	*Aspergillus niger*
A	*Bacillus* sp., *Cyanobacteria, Enterobacter, Escherichia*	*Aspergillus niger*
A+50ml	*Streptomyces* sp., *Cyanobacteria, Corrynebacterium, Escherichia*	*Aspergillus fumigates* sp
A+100ml	*Rhodococcus rhodochrous, Bacillus cereus, Cyanobacteria, Enterobacter, Escherichia*	*Aspergillus niger*
A+150ml	*Bacillus* sp., *Cyanobacteria, Escherichia*	*Fusarium*
A+200ml	*Pseudomonas* sp, *Rhodococcus* sp, *Bacillus sp* ., *Cyanobacteria, Escherichia*	*Fusarum, Chlamydosporum, Pennicillium* sp

Table 2: Biochemical Characteristics of bacterial isolates in Bio-augumented fungi-bacterial effective-microorganismsTreated Soils

Isolate code	**Microscopy**							**Starch Hydrolysis**									**Macroscopy**	**Probable identity**
R	+small Cocci in chains	+	-	+	+	-	+	+	+	-	-	N	A	N	A	AG	Deep yellow round raised	*Staphylococcus epidermidis*
T	+ve chained ellipsoidal rods with white centre	+	+	-	+	+	+	+	+	+	+	A	A	AG	AG	AG	White large round cerrated edge, flat surface	*Bacillus cereus*
A	-ve slender rods	+	+	+	-	+	+	-	-	-	+	AG	A	AG	A	AG	Red small round smooth	*Escherichia hermani*
G	-ve short rods	+	+	+	-	+	+	-	-	+	+	A G	A	N	A	A	Cream Yellow circular flat	*Bacillus*
M	+ve cocci in	+	-	-	+	+	+	+	+	-	-	A	N	N	A	A G	Cream round raised with	*Staphylococcus*
G	-ve single short	+	-	+	-	+	+	-	-	-	-	A G	N	A	A	A	White raised rough surface	*Escherichia coli*
V																	Green small round raised	*Pseudomonas*

Table 3. Characterization and Identification of Bacterial Isolates in BFBE Treated Soils

Isolate code	Bacterial Species	Morphological/cultural Characteristics	Biochemical Reactions
R	*Bacillus*	Rods, short/long, endosphores formed, motile, Gram positive, occur in pairs/chains, colonies are clear/opaque/tarisluscent, milky/creamy/whitishicolourles	Strict aerobes/facultative anaerobes, oxidative/fermentative metabolism/both, usually catalase positive/a few negative, starch hydrolysis positive/sometimes negative, nitrate reduced, acid no gas from glucose, arabinase and mannitol
S	*Corrynebacterium*	Rods, straight/slightly curved, short/long, non-motile, occur singly/pair, Gram positive, colony opaque, yellow/pink/colourless, convex.	Aerobes/facultative anaerobes, oxidative and fermentative metabolism, catalase positive, urease positive/negative, starch hydrolysis weak/negative, most species produce acid no gas from glucose and other sugars. nitrate reduced.
T	*Enterobacter*	Rods, short, motile, Gram negative, colonies clear, milky.	Facultative anaerobes, oxidative and fermentative metabolism, citrate positive, V.P. test positive, catalase positive, citrate positive, nitrate reduced, acid and gas from glucose and other sugars, urease negative/a few positive.
E	*Escherichia*	Rods, moderate/long, occur singly / pairs, motile/a few non- motile, Gram negative, colonies on nutrient agar are clear, creamy	Facultative anaerobes, oxidative and fermentative metabolism, methyl Red positive. indole positive, catalase positive, nitrate reduced, acid and gas from
C	*Staphylococcus*	Cocci, small in diameter, occur singly/pairs/clusters, non- motile, Gram positive, colonies are opaque, yellow/orange/cream/ whitish	Facultative anaerobes, oxidative and fermentative metabolism, Catalase positive, coagulase positive/ negative, urease weak positive/ negative, nitrate reduced, acid from sucrose, maltose, lactose, mannitol.
U	*Streptococcus*	Cocci, small/moderate in diameter, occur in pairs/chains, non-motile, Gram positive, colonies clear, pinkish/ colourless.	Facultative anaerobes, oxidative metabolism, V.P. test positive/ negative, acid from lactose, mannitol.

Table 4: Characterization and Identification of Fungi Isolates in BFBE Treated Soils

M	Green suede/velvety growth with white periphery cream to yellow reverse	Septate hyphae with brached conidiophores bearing phialides coridia are arranged in chains on phialides	*Pennicillium* sp
Q	Flat white fluffy colony with depressed centre light-pink reverse	Septate hyphae bearing conidia laterally to the hyphae	*Apergillus* sp
S	White periphery with greenish centered spores yellow reverse	Septate hyphae with conidiophores bearing coridia.	*Aspergillus* sp
W	lawn like growth with yellow periphery in a spiky leafy form, yellow reverse	Oval shaped budding cells	*Fusarium* sp
	Pink red glistering small round colony with moist surface	Oval shaped budding cells	*Rhodotorula* sp
	Fluffy colony with a distinct rose red, white- yellow reverse White	Oval shaped budding cells	*Fusarium* sp

Table 5: Macroscopic Characterization and Identification of Fungi Isolates in Bio-augumented fungi-bacterial effective-microorganisms Treated Soils

Isolate Code	Macroscopy	Microscopy	Probable Identity
A	White fluffy spores, white reverse	Erect sporangiosphores forming large erminal, globose sporangia	*Mucor sp*
F	White background, blackspores brown reverse	Septate hyphae bearing Conidia	*Aspergillus sp*
G	White flat fluffy, white reverse	Hyphae are aseptate bearing Conida	*Mucor sp*
H	White spores, white reverse	Hyphae are aseptate	*Mucor sp*
L	Dark brown black spores surrounded with white edge,brown reverse	Biserate Coridia Head With phialides borne On septate metulae	*Aspergillus niger*


Rhodotorula sp *Fusarium sp*


3 days old culture of Mucor sp *Aspergillus lentulus*

Discussion

The addition of BFBE for the remediation of soil contaminated with hydrocarbon stimulated a higher population of total heterotrophic bacterial count, this results agrees with the findings of Ene, *et al.,* (2020) which stated that, the combination of soil and activated EM (SO+EMAS) had the highest heterotrophic count compared with the control treatment without the addition of activated EM (NO EMAS), from the results, the addition of activated EM on the various treatments increases the heterotrophic count of bacteria, this result agreed with the findings of Ene, *et al;* (2020). which reported that inoculation with bacterial EM and fungi EM had a stimulating effect to increase the microbial density in soil significantly then water only, the principal activity of EM appears to increase the bio-diversity of soil microflora, thereby increasing plant yield Mohan, (2008). These results is also in accordance with the research findings of Reganold, *et al*; as stated by Ene, *et al;* (2020), state that, environmental pollution problems cannot be solved without using microbial methods and technologies in coordination with agricultural production. This implies that the fate of petroleum products found in soil depends solely on the soil microbial community, as degradation cannot take place without these hydrocarbonoclastic microorganisms. Ene, and Opurum, (2024) revealed that, BFBE contains bacterial and fungi that degrades crude oil in soil. Hydrocarbon utilizing bacterial showed a variation after application, 200ml recorded the highest population. The microbes present in the soil first recognize the oil and its constituent by biosurfactants and bio emulsifiers, and then they attach themselves and use the hydrocarbon present in the petroleum as a source of energy and carbon Bijay, *et al.,* (2012). similar findings was recorded by Ugochukwu, (2023). The hydrocarbon utilizing (hydrocarbonoclastic) bacteria isolated from the crude oil contaminated and bio remediated soils were *Serratia, Pseudomonas, Bacillus, E,Coli, Rhodococcus, Enterbacteria,and Streptococus species* (Table 4). Stimulated biodegradation of crude oil is currently being promoted because it ensures rapid remediation of oil-contaminated ecosystems Okafor, *et al*., (2018). The prevalence of bacillus could be due to the fact that it forms spores, which helps the microorganisms to withstand harsh conditions such as sun drying. The isolation of Bacillus species from crude oil polluted soils could also be due to the ubiquity of the microorganisms. The isolation of *Bacillus, Pseudomonas, Flavobacterium, Serratia, Bacillus* and *Klebsiella* among other bacteria from crude oil polluted lithospheric environment was reported by Okafor, *et al.,* (2016). The fungi isolated from the treated crude oil contaminated soils were *Aspergillus spp, mucus, fusarium, chlamydosporum*, these fungi according to Ene and Opurum., (2024) are fungi that have the potential for bioremediation which are component of effective microorganisms such as *Aspergillus, Saccharomyces spp.* and *Mucor spp*. However, Ugochukwu, (2023) include microbes such as *Mucor sp., Aspergillus niger,* and *Fusarium sp.*

Conclusion

The results from the research have proved that Bioremediation Potential of Bioaugmented Fungi-Bacterial Effective-Microorganisms enhanced soil microbial properties. The results showed that, BFBE has the potential to enhance beneficial microorganisms by breaking the hydrocarbon content in the polluted soil, it also enhances soil bacterial and fungi populations

Recommendations: The use of bioaugmented fungi-bacterial effective-microorganisms is recommended for bioremediation of crude oil polluted soil and enhance microbial diversity in crude oil contaminated soils. bioaugmented fungi-bacterial effective-microorganisms should be used at 100ml to 200ml per liter of water to enhance and improve soil microbial properties.

Reference

Adoki.A. & Orugbani, T. (2007). Influence of nitrogenous fertilizer plants effluents on growth of selected farm crops in soil polluted with crude petroleum hydrocarbons. *Africa. Journal. Agricultural. Research*. 2 (11). 569-573.

Bijay, T., Ajay, K . C., Anish, G. (2012). A Review on Bioremediation of Petroleum Hydrocarbon Contaminants in Soil. *Kathmandu University Journal of Science, Engineering and Technology Vol. 8, No. I, 164-170.*

Chandran, H., Meena, M., & Sharma, K. (2020). Microbial biodiversity and bioremediation assessment through omics approaches. *Frontiers in Environmental Chemistry*, *1*, 570326.

Ene, D. U & Opurum, A. N. (2023). Effectiveness of Effective Microorganisms (EM) for Bioremediation of Hydrocarbon Polluted Soils- A Systematic Review. *Journal of Agriculture, Environmental Resources and Management* ISSN2245-1800(paper) ISSN 2245-2943.

Ene, D. U, Orji, O.A & Nwonuala A.I.(2020). Contributions of Effective Microorganisms and Organic Materials on Microbial Activities. *Agri Res & Tech: Open Access J.* 2020; 25 (2): 556302. DOI: 10.19080/ARTOAJ.2020.25.556302.

Ene, D. U., Ikpe, F. N., & Nwonuala A. I. (2018). Decomposition Rates of Plant Residues and Nutrient Dynamics under Field Conditions in a Humid Tropical Environment.*Direct Research Journal of Agricultural and Food Science.* 6 (5), 121-126,

Guerra A. B., Oliveira J. S., Silva-Portela R. C., Araujo W., Carlos A. C., Vasconcelos A. T. R., et al. (2018). Metagenome enrichment approach used for selection of oil-degrading bacteria consortia for drill cutting residue bioremediation. Environ. Pollut. 235 869–880.

Integrated Bio Products, Certified Manufacturer of EM-1 (2020).

Kleiber,T., Starzyk.,J Górski,R., Sobieralski,K., Siwulski,M., Rempulska, A., Sobiak, A. (2014). The Studies on Applying of Effective Microorganisms (Em) and Crf On Nutrient Contents in Leaves and Yielding of Tomato. *Acta Sci. Pol., Hortorum Cultus 13(1), 79-90*

kpe,F.N., Ndegwe, L.G., Gbaraneh,L.G., Torunama, J.M.A., Williams, T.O., & Larba.A. (2003). Effect of sheep browse diet on fecal matter decomposition and N and P cycling in the humid lowlands of West Africa. Soil Science 168.No.9

Mohan, B. (2008).Evaluation of organic growth promoters on yield of dryland vegetable crops in India.Journal of Organic Systems, 3,23–36.

Okafor, U.C.; Orji M.U.; Nwankwegu A.S.; Anaukwu C.G.; Onuorah S.C.; Archibong E.J.; Obika I.E.; Agu K.C. (2016). Effect Of Chicken Droppings Amendment on Bioremediation of Crude Oil Polluted Soil. *European Journal of Experimental Biology*, 6, 62-68.

Okafor, U.C.; Orji, M.U.; Agu, K.C.; Awah, N.S.; Okeke, B.C.; Okafor, O.I.; Okoro, N.C.N (2016). Bioremediation of Crude Oil-polluted Soil Using Broiler-Chicken droppings. *Journal of Applied and Environmental Microbiology, 4, 75-84.*

Ron, E.Z. and Rosenberg, E. (2001). "Natural roles of biosurfactants." *Environmental Microbiology*, 3(4), 229-236.

Smith, J. W. N., & Harries, N. D. (2014). The life and times of the Sustainable Remediation Forum-UK. What worked, and mistakes to avoid. In: Proceedings of Sustainable Remediation 2016. Montreal, Canada

Sun, P. F., Fang, W. T., Shin, L. Y., Wei, J. Y., Fu, S. F., & Chou, J. Y. (2014). Indole-3-Acetic Acid-producing yeasts in the phyllosphere of the carnivorous plant Drosera indica L. plos one. ; 9(12):e114196.

Ugochukwu, C. O. (2023). Evaluation of the Impact of Crude Oil Contamination on Soil's Physicochemical Characteristics, Micro-flora and Crop Yield. *Tropical Aquatic and Soil Pollution 3(1), 24-35*