



DETERMINATION OF THE EFFECT OF MYCOREMEDIATION ON THE PHYSICOCHEMICAL PROPERTIES OF HYDROCARBON POLLUTED SOILS OF THE NIGER DELTA REGION OF NIGERIA

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ABSTRACT

The study focused on the mycoremediation of hydrocarbon-polluted soils obtained from Niger Delta, Nigeria using indigenous fungal strains. Standard microbiological procedures were adopted for sample collection, identification and preservation; glass wares and media sterilization; sample culturing; fungi isolation, screening, identification and characterization; and physicochemical analysis and the hydrocarbon myco-degradation effects were analyzed using one way ANOVA statistical tool. The results obtained from the study showed that fungal strains (*A. niger*, *A. fumigatus*, *C. brachyspora*, *R. microporus*, and *P. chrysogenum*) that were isolated and utilized are significantly capable of remediating hydrocarbon-polluted soil samples and improved their physicochemical features. The study presented the fairly acidic remediation media of pH range of 6.21 to 6.84 throughout the inoculation period of 60 days. The whole fungal strains pose impact on the understudied physicochemical properties of the soil samples with significant improvements over their initial water holding capacity, total organic carbon content, nitrate content and phosphate content. *C. brachyspora* gave the highest mean remediation efficiency of 69.37% and *P. microspores* gave the least efficiency of 60.67%. It is therefore recommended that the potential of indigenous fungal strains be extensively understudied and applied as cost effective and eco-friendly tool for the bioremediation of hydrocarbon polluted soil sites in Nigeria.

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1.0 INTRODUCTION

The term bioremediation comes from two words, *bios*, meaning life, and *remediate*, meaning to rectify an issue. Bioremediation is a resuscitation process that applies living organisms (green plants, microorganisms, and their relative enzymes), to remove, degrade, mineralize, transform, and detoxify contaminants and hazardous components of the environmental wastes to inert or less-toxic products during the remediation of polluted sites in order to return them to their original state (Azubuike *et al.*, 2016; Environmental Protection Agency, 2016; Sharma, 2020; Donlan and Bauder, 2022). The process entails the biological mechanism of recycling wastes into other forms that can be used and reused by other organisms (Abatenh *et al.*, 2017). The process has been reported in many research works to effectively apply “living things” for the extermination of such environmental problems as contaminated soil and water resources (Goltapeh *et al.*, 2013, Megharaj *et al.*, 2014, Adams *et al.*, 2015, Umeaku *et al.*, 2019).

Every discipline where bioremediation is applied poses unique categorization form but the principle of operation remains relatively the same. Bioremediation as a process is achievable through series of mechanisms that includes biosorption, biodegradation, bioaccumulation, metabolism, biotransformation and detoxification (Humaira, 2018). Donlan and Bauder (2022) maintained that bioremediation potential microbes, through their metabolic activities, have the ability of utilizing chemical pollutants as an energy source, thereby rendering the polluting agents harmless or transforming them to less harmful byproducts. The process is reported by Gomathi *et al.* (2021) to be dependent on the presence of specific microbes like yeast, fungi, or bacteria in the appropriate quantity and blend and in the appropriate optimal environmental conditions.

The processes of extraction, refining, distribution and utilization of hydrocarbon (petroleum) resources have unendingly constituted serious environmental implications and lethal threat to the soil and water biomes. Primarily the effects of petroleum compound pollution on the soil biomes lead to degradation in soil nutritive quality which in turn makes the affected soil not to be useful (Azaizeh *et al.*, 2011). Hydrocarbon pollutants spread rapidly (Vamsi, 2012) and obstruct the supply of oxygen for respiration in aquatic animals and root regions of plants. This ugly condition causes the suffocation and consequent death of organisms living in the soil and water bodies. Guarino *et al.* (2017) reported that oil spillage on land resource makes it unfit for

agricultural or developmental purposes. Hydrocarbon compounds readily clog the root zone and cause the extermination of plants and microorganisms by obstructing oxygen exchange.

The future of the Niger Delta region is extensively threatened by oil pollution and other relative environmental hazard prone situation. The reoccurrence rates command serious attention on ways of optimally salvaging the environmental menace presented by the abounding hydrocarbon pollutants in the area. Mnif *et al.* (2017) holds that such ugly incidences have attracted concerted concerns in Nigeria and other oil sector economy dependent regions across the globe. These concerns have resulted to the present times crusade on dependable or reliable technologies and practices for remediating hydrocarbon compound contaminated environment.

The account of mycoremediation dates back to only a couple of decades ago, and several fungal strains are presently reported as unique and good agents of the process (Merchand, 2017). Mycoremediation is a bioremediation process where fungi are utilized to return a polluted environment to a less polluted state and in more advanced applications encompasses such activities as the addition of carbon based materials to contaminated sites and providing satisfactory condition that tends to promote an increased degradation process (Prakash, 2017). It features the use of fungi's digestive enzymes to degrade and neutralize contaminants like pesticides, hydrocarbons, and heavy metals (Rhodes, 2014). Fungi produce specific enzymes like catalases, laccases and peroxidases, and utilize them to immobilize organic and inorganic pollutants (Morel *et al.*, 2013; Durairaj *et al.*, 2015; Chang *et al.*, 2015; Dell' Anno *et al.*, 2021). Through their physiological activities, fungi tend to be potentially effective in the conversion of organic compounds (hydrocarbon) to energy and environmentally inert biological elements (Singh *et al.*, 2015). Also they have been applied in this feat because of the versatile and suitable nature they present in relation to substrate specifications.

Some of the fungi genera that have been reported to hold great prospects for the bioremediation of hydrocarbon polluted soil include *Aspergillus* (Smita *et al.*, 2012; Al-Hawash *et al.*, 2019; Omer *et al.*, 2020), *Penicillium* (Smita *et al.*, 2012; Govarthanan. 2017; Omer *et al.*, 2020), *Alternaria* (Smita *et al.*, 2012), *Curvularia* (Balaji *et al.*, 2014), *Drechslera* (Dell' Anno *et al.*, 2021), *Fusarium* (Smita *et al.*, 2012; Dell' Anno *et al.*, 2021); *Lasiodiplodia* (Wang *et al.*, 2014), *Mucor* (Dell' Anno *et al.*, 2021), *Rhizopus* (Smita *et al.*, 2012; Hassan, 2014), *Cladosporium*

(Smita *et al.*, 2012), *Bionectria ochroleuca* (Kota *et al.*, 2014), *Trichoderma* (Kota *et al.*, 2014), etc.

This study is therefore undertaken on to determine the effect of mycoremediation on the physicochemical properties of hydrocarbon polluted soils of the Niger Delta region of Nigeria. It specifically expands on the isolation of heterotrophic fungi from the polluted soil samples, characterization of the fungi using morphological and characteristics, screening the ability of fungi to utilize petroleum hydrocarbons, establishing mycoremediation using fungi fungal strains and ascertaining the effects on the soil through physicochemical analysis of the polluted sample before and after remediation.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Methods described by Umeaku *et al.* (2019) was utilized in this study. Three composite soil samples were collected from crude oil polluted soil sites in three different locations of crude oil contamination. The locations included: Ebedei Obi Ogene, Ukwuani in Delta State; Obi-igbo, Oyigbo in River State and Ebekpi, Esit Eket in Akwa Ibom. One sample each was collected from a non-contaminated site in these local government areas to serve as controls. Sterile trowel was used to collect the contaminated soil samples. Collected soil samples were passed through a sterile 2mm sieve into sterile plastic zip-lock bags, labeled properly, put immediately into a cooling box containing ice, transported to the laboratory, and stored in the refrigerator at 4°C.

2.2 Sterilization of Glass wares and Media

Glass wares were sterilized in a hot air oven at 160°C for 1 hour while the media was sterilized by autoclaving at 121°C for 15minutes. All pipettes and other heat-resistant glass wares were wrapped in aluminum foil to protect the items from recontamination during handling and storage before sterilization is carried out at 160°C for 1hr in the hot air oven. 70% ethyl alcohol was used to swab the top of the working bench in the laboratory where the inoculations was carried out (Umeaku, 2019).

2.3 Isolation of Fungi

Collected soil samples transported to the laboratory in a cooling box with ice were stored at the temperature of 4°C until lab process and analysis took place. Soil from each crude oil contaminated site was serially diluted using distilled water. Ten grams of each of the soil samples was weighed and transferred into 250ml flask containing 100ml of distilled water. The solution was mixed properly and allowed to stand for 2 minutes after which the suspension was decanted into another 250ml flask (Cheesbrough, 2000). Then 1ml of each of the soil suspension was serially transferred into a test tube containing 9ml of distilled water. The suspensions were shaken intermittently for 60 seconds. Ten-fold serial dilution was set up from the soil suspension. A loop-full of the one in 10^4 , 10^6 was inoculated on a prepared sabouraud dextrose agar incorporated with chloramphenical and incubated in an inverted position for 5days. Then, different fungal colonies were isolated and sub-cultured on chloramphenical incorporated mineral salt agar (per litre: 2.0g NaNO₃, 0.5g MgSO₄, 0.5g KCL, 0.01g, Fe₂(SO₄)₃H₂O, 0.14g KH₂PO₄, 1.2g K₂HPO₄, 15g Agar, 0.02g yeast extract and pH 7.2) (Umeaku *et al.*, 2019).

2.4 Identification of the Isolates

The attributes outlined in Steven's Mycology Guidebook (1981) were utilized for the microbial identification of the isolates. The isolates were microscopically and macroscopically identified considering their morphological features.

2.5 Screening of the Bioremediation Ability of Isolated Fungi Strains

Fungal isolates which showed optimal growth were further screened for hydrocarbon utilization, using chloramphenical incorporated mineral salt broth containing 1ml of sterile crude oil in a test tube covered with sterile cotton plug. Incubation was done in an incubator for a period of 14 days. One tube which has no organism inside was kept as control. The content of the tubes were monitored for change in optical density and pH for the initial day, 7th and 14th days using spectrophotomer and pH meter respectively (Umeaku, 2019).

2.7 Bioremediation Studies

The bioremediation of the crude oil was carried out in the laboratory using the method described by Omer *et al.* (2020). 100g of soil samples was weighed into sterile cornical flasks. 20mL of

distilled water was added to each flask with 4ml of fungal spore suspension of individual fungal isolates were respectively introduced into the flask and mixed properly. The conical flasks were covered with cotton wool and kept in the incubator at 28°C, stirred on a rotary shaker for sixty days. One was left un-inoculated to serve as the control.

2.7 Physicochemical Analysis

Standard laboratory procedures were adopted for the physicochemical analysis of both unpolluted and polluted samples. The physicochemical parameters that were determined in the collected soil samples include soil pH, moisture content, water holding capacity, total organic carbon content, nitrate content, and phosphate content.

2.7.1 Determination of soil pH

The pH of soil samples was analyzed using an electronic pH meter. 10g of the soil sample was air-dried after sieving through 2-mm mesh size and transferred into a 50ml-beaker containing 20ml of distilled water. The soil suspension was stirred several times for about 30 seconds using a glass rod. The suspension was allowed to stand for 2 minutes using the pH meter, Hana-digital conductivity meter model 98107 was inserted into the partly settled suspension until a steady pH reading is attained (Umeaku *et al.*, 2019).

2.7.2 Determination of soil moisture content

The method described by Musliu and Salawudeen (2012) and Umeaku *et al.* (2019) was adopted to evaluate the soil moisture content in order to determine the amount of moisture in the soil sample. Six covered petri dishes were oven dried at 105°C for 1 hour, allowed to cool and weighed separately, recording the weight respectively. 10g from each of the soil samples were weighed into each petri dish and their initial weights taken. The petri dishes containing the soil samples were transferred into oven with lid off to dry for 24 hours. The petri dishes containing the samples were transferred to a desiccator before weighing again until a constant weight is obtained. The loss in weight was calculated using the formula below:

$$\text{Moisture content (MC) (\%)} = \frac{W_2 - W_3}{W_3 - W_1} \times 100 \quad (1)$$

Where: W_1 = weight of empty petri dish, (g)

W_2 = weight of petri dish + moist soil, (g)

W_3 = weight of petri dish + oven dry soil, (g)

2.7.3 Determination of water holding capacity

Six small plastic containers with one opened end and closed end were used. A medium sized nail was used to make holes at the closed end of the containers. Whatman's No 1 filter paper that sized the perforated end was placed on the inside of the sealed end through the opened end. The filter paper was moistened with a jet of distilled water from a wash bottle. The weight of the container with the wet filter paper was recorded. Then the container was filled with oven dried soil sample of 24 hours compacted by dropping the container from a height until the soil surface becomes level. The weight is taken again and recorded. The container was put in a bowl containing water until the surface of the soil gets moistened. The container was removed and put in an empty Petri dish to allow the excess water to drain off. The container was then removed and weighed (Musliu and Salawudeen, 2012; Umeaku *et al.*, 2019). The quantity of moisture retained per unit weight of the oven dried soil contained in each of the container was determined using the formula below:

$$M_{ds} = W_2 - W_1 \quad (2)$$

$$M_{ss} = W_3 - W_1 \quad (3)$$

$$M_w = M_{ss} - M_{ds} \quad (4)$$

$$\text{Percentage water holding capacity} = \frac{M_w}{M_{ss}} \times 100 \quad (5)$$

Where W_1 = Weight of plastic container with filter paper, (g)

W_2 = Weight of plastic container with filter paper and dry soil, (g)

W_3 = weight of plastic container and wet soil, (g)

M_{ds} = Mass of dry soil, (g)

M_{ss} = Mass of saturated soil, (g)

M_w = Mass of water contained in saturated soil, (g)

2.7.4 Total organic carbon content

The Walkley-Black method was used to determine the total organic content of the soil samples. 2g of the sample was treated with 5ml of 0.4M potassium dichromate solution ($K_2Cr_2O_7$) followed by addition of 10 ml of concentrated sulfuric acid. The mixture was

gently swirled and left at room temperature in a fume cupboard for 16-18 hours and then, 100 ml of distilled water was added to the mixture. The excess of dichromate was back- titrated potentiometrically with the use of standard 0.5M ferrous ammonium sulfate ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$) solution (Walkley and Black, 1934). Blank titration of the acidic dichromate with ferrous ammonium sulfate solution was performed also. Organic carbon content in the sample was calculated as:

$$\text{Organic carbon, \%} = \frac{(B - S) \times M \times 0.003 \times F \times 100}{\text{Weight of sample}} \quad (6)$$

Where: B = The volume of ferrous solution used in the blank titration

S = The volume of ferrous solution used in the sample titration,

M = Molarity of ferrous ammonium sulfate

F = Correction factor, 1.3

2.7.5 Nitrate content

Nitrate of the solutions obtained from the soil samples were measured by spectrophotometric methods. 50ml of the extracted soil sample was pipetted into a porcelain dish and evaporated to dryness on a hot water bath. 2ml of phenol disulphonic acid was added to dissolve the residue by constant stirring with a glass rod. Concentrated solution of sodium hydroxide and distilled water was added with stirring to make it alkaline. This was filtered into a Nessler's tube and made up to 50ml with distilled water. The absorbance was read at 410nm using a spectrophotometer after the development of colour. The concentration of the samples was determined using the expression:

$$\text{Concentration of sample} = \frac{\text{Absorbent of sample}}{\text{Absorbent of standard}} \times \text{concentration of standard} \quad (7)$$

2.7.6 Phosphate content

Phosphorus content in each sample was determined using the Olsen method. 2g of the sample was weighed with the aid of digital weighing balance into crucible, covered and inserted into the muffle furnace at the temperature of 550°C for 2hours, allowed to cool and then 20ml of 20% sulphuric acid was measured into 250ml beaker containing the sample, stirred and digested on hot plate at the temperature of 80°C for 10minutes, allow to cool and filtered with Whatman no.4 filter paper and the volume make up to 100ml with distilled water and stored in a reagent bottle for phosphate analysis. 100ml of the individual filtrate soil samples were poured into a

250ml conical flask. The same volume of distilled water (serving as control) was also measured into another conical flask. 1ml of 18M H₂S0₄ and 0.89g of ammonium persulphate were added to both conical flasks and gently boiled for 1 ½ hrs, keeping the volume of 25-50cm³ with distilled water at temperature of 80⁰C. It was then allowed to cool in a dessicator and the one drop of phenolphthalein indicator was added and after neutralized to a faint pink colour with the aid of 2M NaOH solution. The pink colour was discharged by drop wise addition of 2M HCl, and the solution made up to 100ml with distilled water. For the colorimetric analysis, 20ml of the sample was pipette into test tubes, 10ml of combined reagent was added, shaken and left to stand for 10mins before reading the absorbance at 690nm on a spectrophotometer, using 20ml of distilled water plus 1ml of the reagent as reference (Olsen *et al.*, 1954).

2.8 Data Analysis

The major statistical technique that was used in this study for the analysis of the collected data is Analysis of Variance (ANOVA). In this study, only the One-way ANOVA was used to analyze generated data.

3.0 RESULTS AND DISCUSSION

3.1 Results

Table 1: Microscopically and macroscopically identified fungi strains from collected samples

Sample	Identified microorganism
AHPS	<i>Aspergillus niger</i>
	<i>Rhizopus microspores</i>
	<i>Aspergillus fumigates</i>
	<i>Mucor spp</i>
	<i>Cladosporium brachyspora</i>
RHPS	<i>Aspergillus niger</i>
	<i>Aspergillus fumigatus</i>
	<i>Penicillium chrysogenum</i>
DHPS	<i>Aspergillus fumigatus</i>
	<i>Aspergillus niger</i>
	<i>Cladosporium brachyspora</i>
	<i>Rhizopus microspores</i>

Legend:

AHPS – Akwa Ibom hydrocarbon-polluted soil

RHPS – Rivers hydrocarbon-polluted soil

DHPS – Delta Hydrocarbon-polluted soil

Table 2: Optical density measurement at 540nm and pH

Fungi isolates	Day 1		Day 7		Day 14	
	Optical density	pH	Optical density	pH	Optical density	pH
<i>Aspergillus niger</i>	0.217	6.770	0.386	6.350	0.497	6.850
<i>Cladosporium brachyspora</i>	0.202	5.880	0.269	6.030	0.386	6.880
<i>Rhizopus micropores</i>	0.198	6.560	0.289	7.025	0.366	6.460
<i>Penicillium chrysogenum</i>	0.301	6.370	0.359	6.440	0.401	6.971
<i>Aspergillus fumigatus</i>	0.311	6.681	0.389	6.421	0.486	6.523

Table 3: Physicochemical properties of un-inoculated hydrocarbon-polluted soil samples

Sample	Tempera -ture (°C)	pH	Moisture content (%)	Water holding capacity (%)	Total Organic Carbon (%)	Nitrate (mg/kg)	Phosphate (mg/kg)
AHPS	28.20	5.10	9.00	19.70	1.73	2.13	1.04
RHPS	27.90	6.01	9.00	18.00	2.15	1.98	1.51
DHPS	28.00	5.80	2.00	20.00	1.98	2.09	1.37

Table 4: Mean physicochemical properties of Akwa Ibom State fungal strains remediated soil samples after 60 days

Samples	Tempe- rature (°C)	pH	Water holding capacity (%)	Total Organic Carbon (%)	Nitrate (mg/kg)	Phosphate (mg/kg)
AHPS (A. niger inoculated)	32.45	6.29	20.17	3.20	6.68	3.41
AHPS (Cladosporium inoculated)	30.38	6.73	21.26	3.05	5.28	2.78
AHPS (Penicillium inoculated)	32.87	6.51	28.86	4.02	6.98	3.11
AHPS (Rhizopus inoculated)	30.32	6.21	20.16	2.95	5.11	3.18
AHPS (A. fumigatus inoculated)	28.25	6.82	20.00	2.98	6.28	3.88

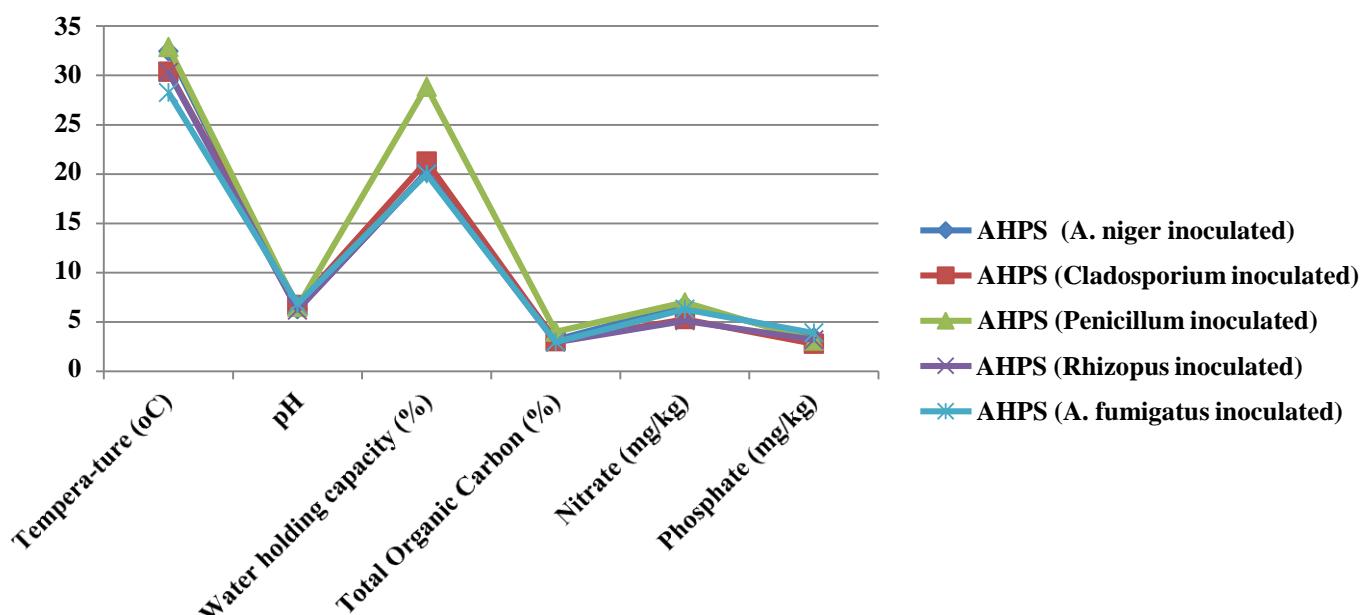


Fig. 1: The physicochemical characteristics of fungal strain inoculated AHPS samples

Table 5: Mean physicochemical properties of Rivers State fungal strains remediated soil samples after 60 days

Samples	Tempe- rature (°C)	pH	Water holding capacity (%)	Total Organic Carbon (%)	Nitrate (mg/kg)	Phosphate (mg/kg)
RHPS (A. niger inoculated)	30.98	6.43	18.77	4.43	7.16	3.95
RHPS (Cladosporium inoculated)	29.31	6.49	20.11	3.93	6.74	3.88
RHPS (Penicillium inoculated)	30.82	6.56	26.22	3.98	6.66	4.16
RHPS (Rhizopus inoculated)	29.98	6.22	22.57	3.03	5.24	3.55
RHPS (A. fumigatus inoculated)	29.30	6.84	16.98	3.94	6.84	3.66

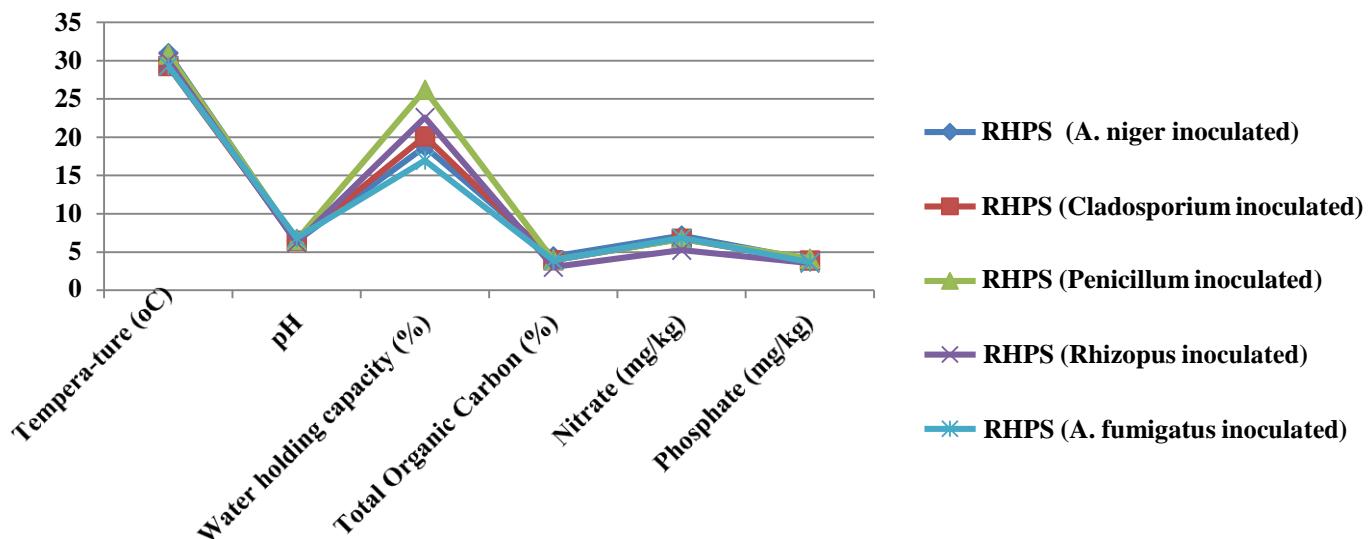


Fig. 2: The physicochemical characteristics of fungal strain inoculated RHPS samples

Table 6: Mean physicochemical properties of Delta State fungal strains remediated soil samples after 60 days

Samples	Tempe- rature (°C)	pH	Water holding capacity (%)	Total Organic Carbon (%)	Nitrate (mg/kg)	Phosphate (mg/kg)
DHPS (A. niger inoculated)	33.18	6.72	23.12	3.92	5.83	3.09
DHPS (Cladosporium inoculated)	28.14	6.77	21.14	3.62	4.93	3.11
DHPS (Penicillium inoculated)	30.27	6.63	29.46	4.16	6.33	4.25
DHPS (Rhizopus inoculated)	29.10	6.72	23.24	3.57	5.03	3.79
DHPS (A. fumigatus inoculated)	28.60	6.60	21.23	3.42	4.92	3.13

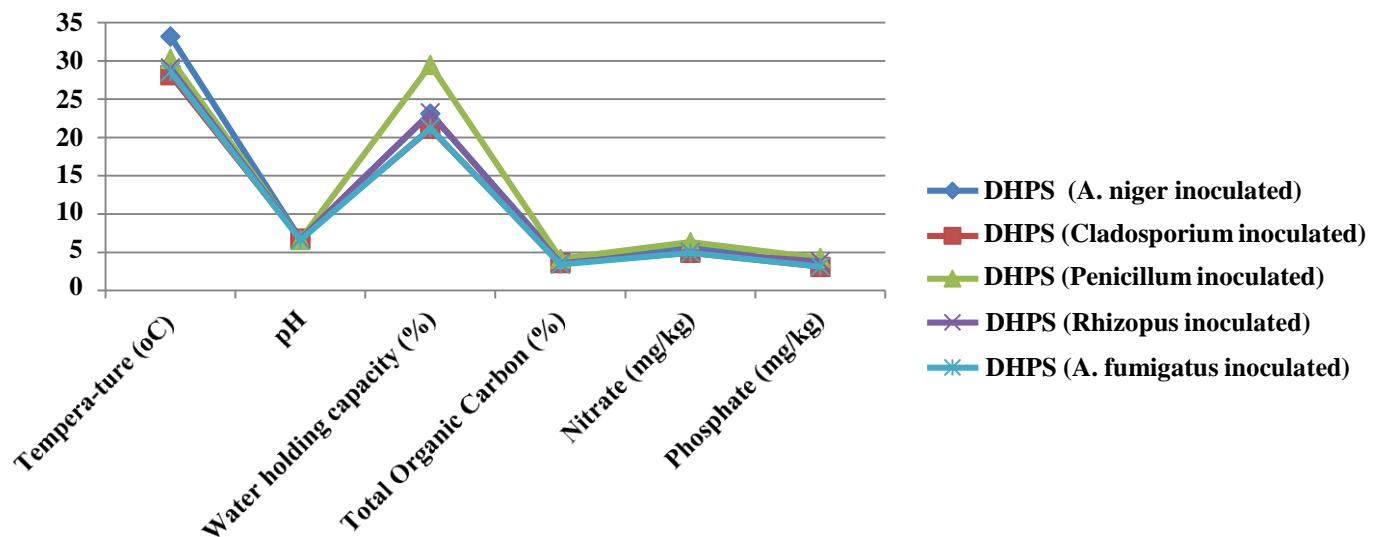


Fig. 3: The physicochemical characteristics of fungal strain inoculated DHPS samples

Table 7: ANOVA of the mean effect of fungal strains remediation on physicochemical properties of the soil samples

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1936.72	5.00	387.34	881.05	5.87E-15	3.11
Within Groups	5.28	12.00	0.44			
Total	1942.00	17.00				

3.2 Discussion

After the inoculation of the samples on chloramphenical incorporated Sabouraud Dextrose Agar and incubating for 5 days, the isolated fungal colonies were further sub-cultured on Mineral Salt Agar incorporated with chloramphenicol in order to obtain pure culture. The isolates were macroscopically and microscopically identified using the morphological attributes outlined in the Steven's Mycology Guidebook (Table 1). The screening test carried out on the extracted indigenous fungi for 14 days on mineral salt medium supplemented with crude oil as the only carbon source showed that the isolated and identified fungal strains (*A. niger*, *A. fumigatus*, *C. brachyspora*, *Rhizopus microporus*, and *Penicillium chrysogenum*) portrayed active potentials in utilizing crude oil for their growth (Table 2). Table 3 showed the respective results obtained from selected physicochemical properties (pH, temperature, water holding capacity, total organic carbon content, nitrate content and phosphate content) analyses of samples obtained from the hydrocarbon polluted soil samples from Akwa Ibom, Rivers and Delta States.

Tables 4, 5 and 6 showed the results for the respective physicochemical properties analyses of *A. niger*, *A. fumigatus*, *C. brachyspora*, *Rhizopus microporus*, and *Penicillium chrysogenum* inoculated soil samples. The results showed that the fungal bioremediation processes presented the pH of fairly acidic range of 6.21 to 6.84 media which was related to Ahmad *et al.* (2019) position of pH of the range of fairly acidic (6.82) to acidic (5.70). There is rise in temperature through the remediation application which supports the report that microbial activities generate heat and cause an increase in soil temperature. The whole fungal strains remedied soil sample showed significant improvements over their initial water holding capacity, total organic carbon content, nitrate content and phosphate content. These recorded improvements sustain fungal strains as viable bioremediation tool for both agricultural and other environmental pollution detoxification processes. Figures 1, 2, and 3 showed the comparative outlook of the effects of the fungal strains remediation and their trends indicated that the bioremediation processes followed relatively the same pattern across all the understudied physicochemical parameters.

Tables 7 showed the remediation effects of the fungal strains. With higher F_{cal} value obtained over the F_{tab} values, the use of indigenously isolated fungal strains where of significant effects to the remediation of hydrocarbon-polluted soils at a probability of 5%. The application of mycoremediation improved the physicochemical properties positively.

4.0 CONCLUSION

The study determined the effects of mycoremediation on the physicochemical properties of hydrocarbon polluted soils of the Niger Delta Region. Standard microbiological procedures and instrumentations were utilized for the fungi-primed remediation process and statistical tools were utilized in the result presentation and analysis. The results show that the physicochemical parameters of the Fungal strains inoculated soil samples maintained improved features than the values obtained from the samples of the hydrocarbon-polluted sites, except for their mean temperatures that were relatively the same. The processes presented a fairly acidic pH range of 6.21 to 6.84. The whole fungal strains remedied soil sample showed significant improvements over their initial water holding capacity, total organic carbon content, nitrate content and phosphate content. *C. brachyspora* gave the highest mean remediation efficiency of 69.37% and *P. microspores* gave the least efficiency of 60.67%. The ANOVA results further showed that the use of indigenously isolated fungal strains where of significant effect to the remediation of

hydrocarbon-polluted soils at a probability of 5%. In principle, the study bridges the gap of the relatively little or no literal evidences on fungal strains remediation – a novel alternative technique that is cost effective, efficient and eco-friendly.

REFERENCES

Abatenh, E., Gizaw, B., Tsegaye, Z. and Wassie, M. (2017). The Role of Microorganisms in Bioremediation. *A Review. Open Journal of Environmental Biology*, **2**(1): 038-046.

Adams, G. O., Fufeyin, P. T., Okoro, S. E. and Ehinomen, I. (2015). Bioremediation, Biostimulation and Bioaugmentation: A Review. *International Journal of Environmental Bioremediation & Biodegradation*, **3**(1): 28-39.

Al-Hawash, A. B., Zhang, X. and Ma, F. (2018). Removal and biodegradation of different petroleum hydrocarbons using the filamentous fungus *Aspergillus sp.* RFC-1. *Wiley-Microbiology Open*, 14 pages.

Azaizeh, H., Castro, P. M. L. and Kidd, P. (2011). Biodegradation of organic xenobiotic pollutants in the rhizosphere. *Organic Xenobiotic Plants*, **8**:191-215.

Azubuike, C. C., Chikere, C. B. and Okpokwasili, G. C. (2016). Bioremediation techniques—classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*, **32**:180.

Balaji, V., Arulazhagan, P. and Ebenezer, P. (2014). Consortia enriched from petroleum contaminated soil and oil seeds. *Journal of Environmental Biology*, **35**(3):521-529.

Chang, Y. T., Lee, J. F., Liu, K. H., Liao, Y. F., and Yang, V. (2015). Immobilization of fungal laccase onto a nonionic surfactant-modified clay material: application to PAH degradation. *Environmental Science Pollution Resources*, **20**:15

Cheesbrough, M. (2000). Biochemical testing of Microorganisms in: *Medical Laboratory Manual for Tropical Countries. Vol 11: Microbiology*. Cambridge University Press, pp 58–69.

Dell' Anno, F., Rastelli, E., Sansone, C., Brunet, C., Ianora, A., Dell' Anno, A. (2021). Bacteria, Fungi and Microalgae for the Bioremediation of Marine Sediments Contaminated by Petroleum Hydrocarbons in the Omics Era. *Microorganisms*, **9**: 1695.

Donlan, D. L. and Bauder, J.W. (2022). A General Essay on Bioremediation of Contaminated Soil. MSU Extension Water Quality Program, Montana State University. Online article.

Durairaj, P., Malla, S., Nadarajan, S. P., Lee, P. G., Jung, E., Park, H. H., Kim, B. G., and Yun, H. (2015). Fungal cytochrome P450 monooxygenases of *Fusarium oxysporum* for the synthesis of hydroxy fatty acids in engineered *Saccharomyces cerevisiae*. *Microbiology Cell Factories*, **14**:1–16.

Environmental Protection Agency – EPA (2016). United States Environmental Protection Agency. <https://www3.epa.gov/>

Goltapeh, E. M., Danesh, Y. R. and Varma, A. (2013). Fungi as Bioremediators. *Soil Biology*, **32**. #Springer-Verlag Berlin Heidelberg.

Gomathi, T., Saranya, M., Radha, E., Vijayalakshmi, K. Prasad, P. S. and Sudha, P. N. (2021). Bioremediation: A Promising Xenobiotics Cleanup Technique. John Wiley & Sons Ltd. *Encyclopedia of Marine Biotechnology*, **5**(1): 3139-3172.

Guarino, C., Spada, V. and Sciarrillo, R. (2017). Assessment of three approaches of bioremediation (natural, attenuation, landfarming and bioaugmentation — Assisted land farming) for a petroleum hydrocarbons contaminated soil. *Chemosphere*, **170**:10-16.

Humaira, N. (2018). A study on various aspects of bioremediation with microbes as a tool of effective waste management. A B. Sc. Thesis submitted to Department of Mathematics and Natural Sciences, BRAC University, Bangladesh. 32 pages

Kota, M., Husaini, A. A. S. A., Zulkharnain, A., and Roslan, H. (2014). Bioremediation of Crude Oil by Different Fungal Genera. *Asian Journal of Plant Biology*, **2**(1): 11–18.

Megharaj, M., Venkateswarlu, K. and Naidu, R. (2014). Bioremediation. Encyclopedia of Toxicology (Third Edition). <https://www.sciencedirect.com/science/article/pii/B9780123864543010010>

Merchand, C. (2017a). Phytoremediation of soil contaminated with petroleum hydrocarbons and trace elements. Linnaeus University Dissertations, Nr 279/2017. linnaeus university press. 82 pages. ISBN: 978-91-88357-63-2.

Mnif, S., Chebbi, A., Mhiri, N., Sayadi, S. and Chamkha, M. (2017). Biodegradation of phenanthrene by a bacteria consortium enriched from Sercina oilfield. *Process Safety and Environmental Protection*, **107**: 44-53.

Morel, M., Meux, E., Mathieu, Y., Thuillier, A., Chibani, K., Harvengt, L., Jacquot, J. P., and Gelhaye, E. (2013). Xenomic networks variability and adaptation traits in wood decaying fungi. *Microbial Biotechnology*, **6**: 248–263.

Musliu, A. and Salawudeen, W. (2012). Screening and isolation of the soil bacteria for ability to produce antibiotics. *European Journal of Applied Sciences*, **4**(5): 211-215.

Omer, A. A., Riyad, A. F. and Mazin, N. F. (2020). Use of fungi in bioremediation of contaminated sites with hydrocarbons. *Plant Archives*, **20** (2): 1406-1410

Prakash, V. (2017). Mycoremediation of Environmental Pollutants. *International Journal of ChemTech Research*, **10**(3): 149-155.

Rhodes, C. J. (2013). Applications of bioremediation and phytoremediation. *Science Progress*, **96**(4), 417-427.

Sharma, I. (2020). Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects. <https://www.intechopen.com/chapters/70661>

Singh, M., Srivastava, P. K., Verma, P. C., Kharwar, R. N., Singh, N. and Tripathi, R. D. (2015). Soil fungi for mycoremediation of arsenic pollution in agriculture soils. *Journal of Applied Microbiology*, **119**: 1278-1290.

Smita, C., Jyoti, L., Vandana, S. and Chetan, S. (2012). Assessment of Diesel Degrading Potential of Fungal Isolates from Sludge Contaminated Soil of Petroleum Refinery, Haryana. *Research Journal of Microbiology*, **7**(3):182-190.

Stevens R. B. (1981). Mycology Guidebook. Mycology Guidebook Committee, Mycological Society of America, University of Washington Press, Seattle

Umeaku, C. N. (2008). Biochemical Testing of Microorganisms in: Laboratory Practice for Tertiary Institutions. Odus Press, Enugu. ISBN 978-38379-7-4

Umeaku, C. N., Emmyegbe, I. O., Ukoha, C. C., Ezenwa, S. E., Chris-Umeaku, C. I. (2019). Biodegradation of Crude Oil-Polluted Soil by Bacterial Isolates from Nigeria. *Frontiers in Environmental Microbiology*, **5**(1):14-28.

Vamsi, K. G. (2012). Biodegradation of Petroleum Hydrocarbons. M. Tech. thesis, Department of Chemical Engineering National Institute of Technology Rourkela, Odisha – 769008.137 pages.

Walkley, A. and Black, I. A. (1934). An Examination of Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration *Methodology in Soil Science*, **37**:29-37.

Wang, C., Liu, H., Li ,J., and Sun, H. (2014). Degradation of PAHs in soil by *Lasiodiplodia theobromae* and enhanced benzo[a]pyrene degradation by the addition of Tween-80. *Environ Science and Pollution Research International*, **21**(18):10614-25.