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CULTURAL CHARACTERIZATION OF FUNGI ISOLATED FROM OIL CONTAMINATED SOILS

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ABSTRACT
Application of Fungi for effective removal of hydrocarbon contamination from soil is being considered as the better option when it comes to biodegradation. Other method like physical and chemical bioremediation leads to production of toxic compounds and these methods are not cost effective. In the present study, soil samples from four different oil contaminated soils were assessed for any recovery of fungi present. Cultural characterization was used as preliminary identification using keys. Initial isolation from the oil contaminated soil was done using potato dextrose agar. Colonies were observed and characterized morphologically. The isolates were grown at varied temperatures and pH. Eight fungal isolates were recovered from polluted soils namely, *Trichoderma viride, Trichoderma spirale, Neosartorya pseudofischeri, Neosartorya aureola, Aspergillus flavus, Aspergillus terreus, Penicillium griseofulvum* and *Trichoderma longibrachiatum*. The optimum growth temperature range for the eight fungi was 30^{o}C and 40^{o}C. There was no growth at 50^{o}C for all isolates except some slight growth by *Aspergillus flavus*. Optimum growth at pH 7 and pH 9 and poor growth at pH 5 was noted. This study will contribute to the database on locally available fungal diversity and their ecology.
Key Words. Fungi, Bioremediation, oil contamination, biodegradation

1.0 INTRODUCTION

Petroleum products are extensively widespread all over the world and their intensive use is strongly connected to heavy discharge of hydrocarbons into the environment (Winkelmann *et al.*, 2009). A major concern for petroleum hydrocarbon bioremediation is the presence of heavy compounds such as polycyclic aromatic hydrocarbons (PAHs. These heavy hydrocarbon constituents are considered potential health risks due to their possible carcinogenic and mutagenic actions (Baheri and Meysami, 2002). Moreover, lubricant oil can persist for more than six years in some ecosystems, resulting in chronic problems to the biota (Burns *et al.*, 1994). Widely use of petroleum hydrocarbon in factories leads to pollution caused by production processes. Pollution occur both in marine and aquatic environment for example harbors, but also in the terrestrial environment, mostly near factories or tank stations. Soil pollution is not considered as a big environmental problem due to the restriction of the pollution to a small local area. Local areas polluted with petroleum are so numerous (Hoeppel and Hinchee, 1991). It is therefore important to develop techniques to efficiently clean these areas. In the terrestrial environment, the contaminating organic compounds first migrate downward in the soil due to gravity. Lighter compounds go further spreading in the layer above the ground water, while the heavier parts continue their way downwards up to the water table. Some compounds can be soluble in the water table, and thus being transported horizontally. This is of interest for soil pollution, because it causes spreading of the pollution and creates the possible entering of organic compound in the ground water used for a variety of purposes (Qin and Huang, 2009). Oil is Niger Delta is lost because of the geographical terrain of which is mostly a mangrove swamp and marsh. The soft flowing mud of the swamps and prop roots of the mangrove trees are usually the natural obstacles during oil recovery efforts in this region; through which heavy machinery cannot be moved for oil recovery efforts. Bioremediation is therefore.a good answer to the removal of oil spilled in these areas and the best means of remediation in such ecosystems (Azaiki, 2009).

2.0 Materials and Methods

2.1 Isolation

Collected soil samples were homogeneously mixed and carefully sorted to remove stones and other unwanted soil debris using a 2.5 mm sieve. The PDA media was autoclaved at 121^{o}C for 15, min allowed to cool and 20ml dispensed aseptically on the sterile disposable petri dishes. One gram of each sorted soil sample was homogenously mixed with 1 drop of Tween 80 to enhance biodegradation and was later sprinkled onto the Potato Dextrose Agar (PDA) media and incubated for 7 days at 30^{o}C. Ampliclox 25mg/l was added to the media after autoclaving to prevent contamination by bacteria. The pure colonies were selected based on morphological characteristics (Aneja, 2005).

2.2 Cultural characterization

To purify the fungal isolates, the cultures were carefully and aseptically sub cultured on Potato Dextrose Agar (PDA) and stored on PDA slants for future use. The fungal isolates were characterized on the basis of cultural characteristics and morphological characteristics including spore type, mycelia and other fruiting bodies in a lactophenol cotton blue wet mount by compound microscope at ×100. Observed characteristics were recorded and compared with the established identification key by Barnett and Hunter (1972).

2.3 Fungal mycelial growth at different temperatures and pH

A 10mm mycelia growth disc was inoculated on PDA media and incubated at 30 ℃, 40 ℃ and 50 ℃ separately for 7 days in triplicates. The radial growth in mm for the colonies was measured and recorded. The PDA media was prepared separately according to manufacturer's instructions in conical flasks. The pH of each set of experiments was adjusted to 5.0, 7.0 and 9 with a pH meter using HCl or NaOH. The media was autoclaved, cooled and dispensed in about 20ml quantities in petri dishes. The petri dishes containing a 10mm mycelia growth disc were incubated at 30 ℃ for a period of 7 days and the radial growth measured in millimeters and recorded (Venosa and Zhu 2003).

2.4 Statistical analysis

Different samples from the garages represented the treatments. There were three replications for every treatment. Results were compared using one-way Analysis of Variance (ANOVA). This was done to establish if differences ($p<0.05$) were significant between individual treatments. The analysis was done using SPSS version 17.

3.0 Results

3.1 Isolation and morphological characterization of fungi

A total of eight fungal isolates were isolated from oil contaminated soils using Potato Dextose Agar. Characterization was based on classical macroscopic techniques of color, margin and elevation of the pure colonies. Most colonies were able to grow within 4-7 days of incubation at 30 ℃. The isolates exhibited different colony characteristics. Three isolates had curled margin, two had entire margin while the rest were undulate and filamentous. The isolates had raised fluffy elevation. The reverse colour was mostly creamish while the obverse varied for each isolate (Table 1and Plate 1-5).

Table 1: Cultural characteristics of fungi isolated from oil contaminated soils.

ISOLATE CODE	ISOLATE IDENTITY	COLOUR (OBVERSE)	COLOUR (REVERSE)	MARGIN	ELEVATION
1	*Trichoderma viride*	Cream white	Cream	Curled	Raised fluffy
4	*Trichoderma spirale*	Grey brown	Dark brown	Entire	Raised fluffy
5	*Neosartorya pseudofischeri*	Granular white	Cream	Filamentous	Raised fluffy
12	*Neosartorya aureola*	White	Cream yellow	Entire	Medium fluffy
13	*Aspergillus flavus*	Green	Cream	Curled	Raised
14	*Aspergillus terreus*	Pinkish brown	Cream green	Curled	Raised
18	*Penicillium griseofulvum*	Dark green	Cream green	Entire	Raised fluffy
19	*Trichoderma longibrachiatum*	Cream white	Cream	Undulate	Raised fluffy

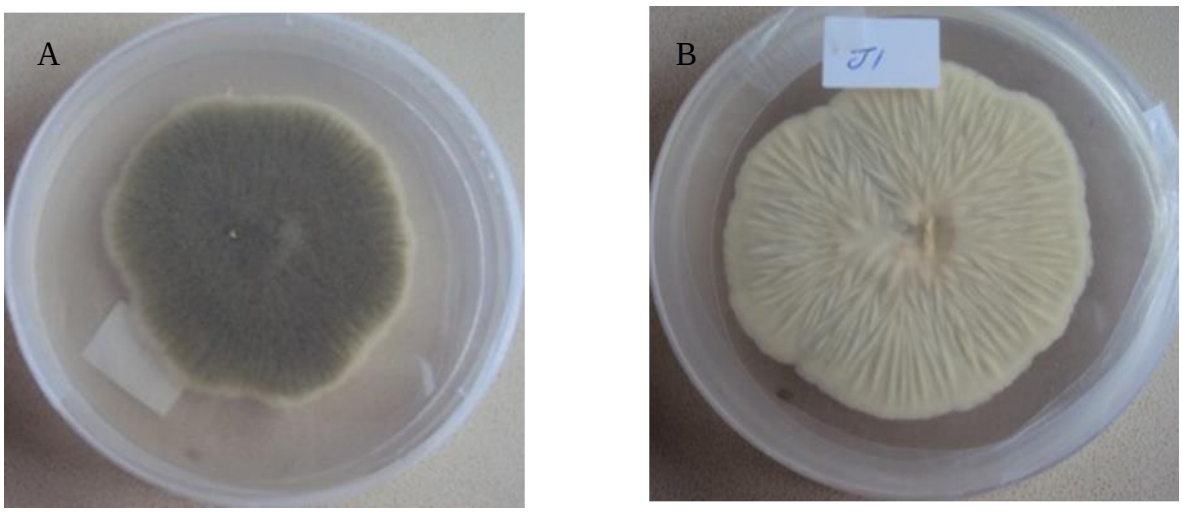

Plate 1: Culture plate showing A: Obverse of Aspergillus flavus on PDA media, B: Reverse of Aspergillus flavus on PDA media.

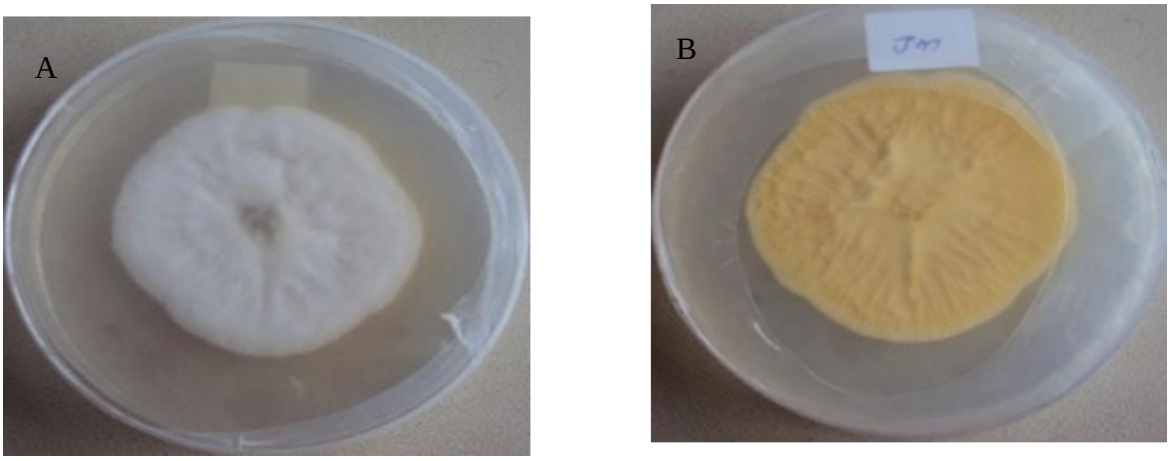

Plate 2: Culture plates showing A: Obverse of *Neosartorya aureola* on PDA media, B: Reverse of *Neosartorya aureola* on PDA media.

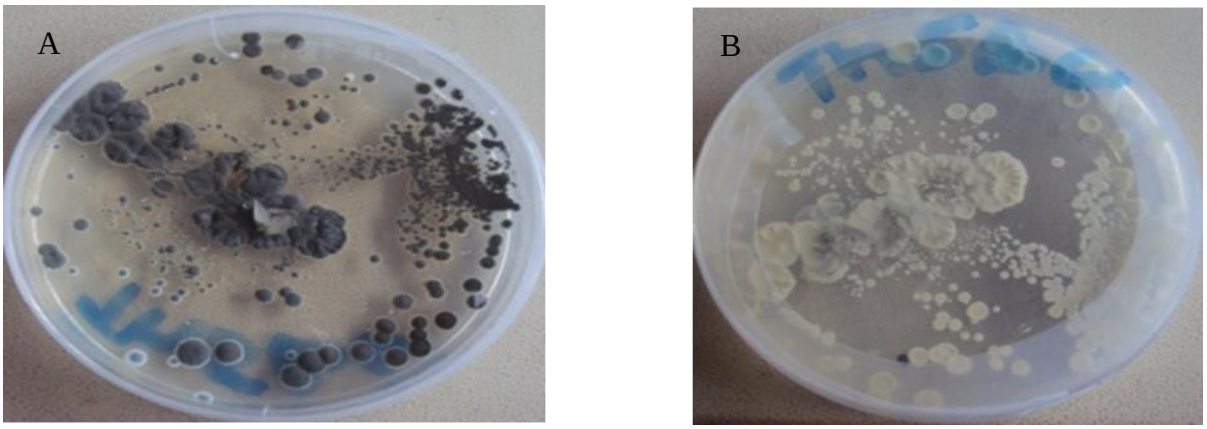

Plate 0: Culture plate showing A: Obverse of *Penicillium griseofulvum* on PDA media, B: Reverse of *Penicillium griseofulvum* on PDA media.

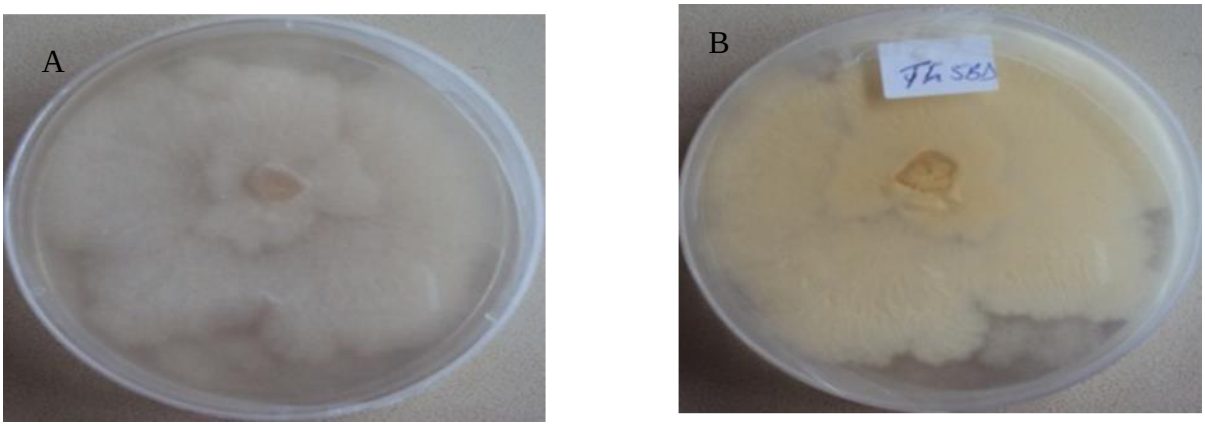

Plate 4: Culture plate showing A: Obverse of *Trichoderma viride* on PDA media, B: Reverse of *Trichoderma viride* on PDA media.

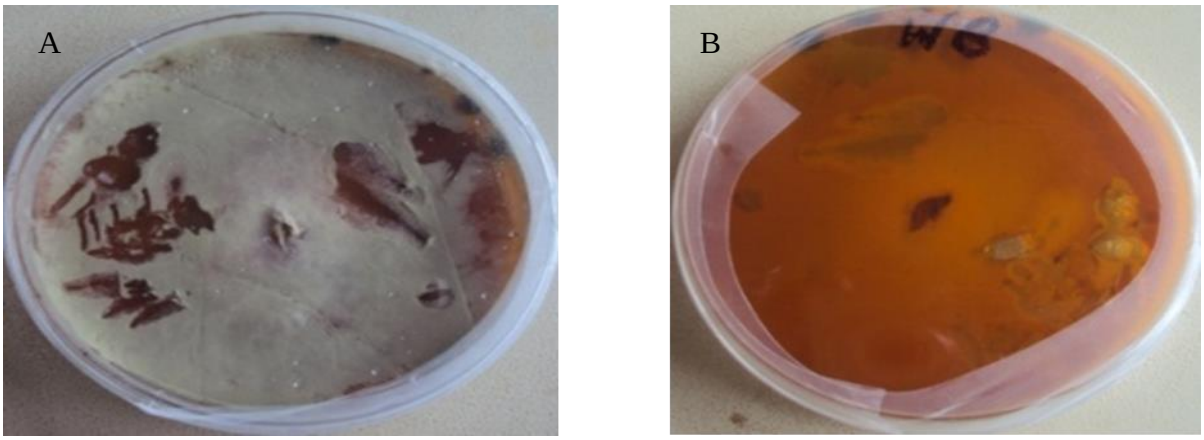

Plate 5: Culture plate showing A: Obverse of *Trichoderma spirale* on PDA media, B: Reverse of *Trichoderma spirale* on PDA media.

3.2 Fungal mycelial growth at different temperatures

When different organisms were grown at different temperatures, they showed different radial growths. *Trichoderma spirale* had optimal growth at 30°C and poor growth at 40°C. There was no growth at 50°C. *Trichoderma longibrachiatum* had optimal growth at 30°C and no growth at 40°C and 50°C. *Neosartorya pseudofisheri* had optimal growth at 30°C and minimal growth at 40°C. No growth was observed at 50°C. *Penicillium griseofulvum* had optimal growth at 40°C and minimal growth at 30°C. No growth was observed at 50°C. *Trichoderma viride* grew only at 30°C and no growth at 40°C and 50°C. *Aspergillus flavus* grew at all temperatures. Optimal growth was observed 40°C, minimal growth at 30°C and poor growth at 50°C. *Aspergillus terreus* had optimal growth at 40°C, and minimal growth at 30°C. No growth was observed at 50°C. *Neosartorya aureola* had optimal growth at 30°C and a very minimal growth at 40°C. No growth was observed at 50°C. Therefore, there was no significance difference $p > 0.05$, 0.997973 on radial growth for fungal isolates at three different temperatures (Fig. 1).

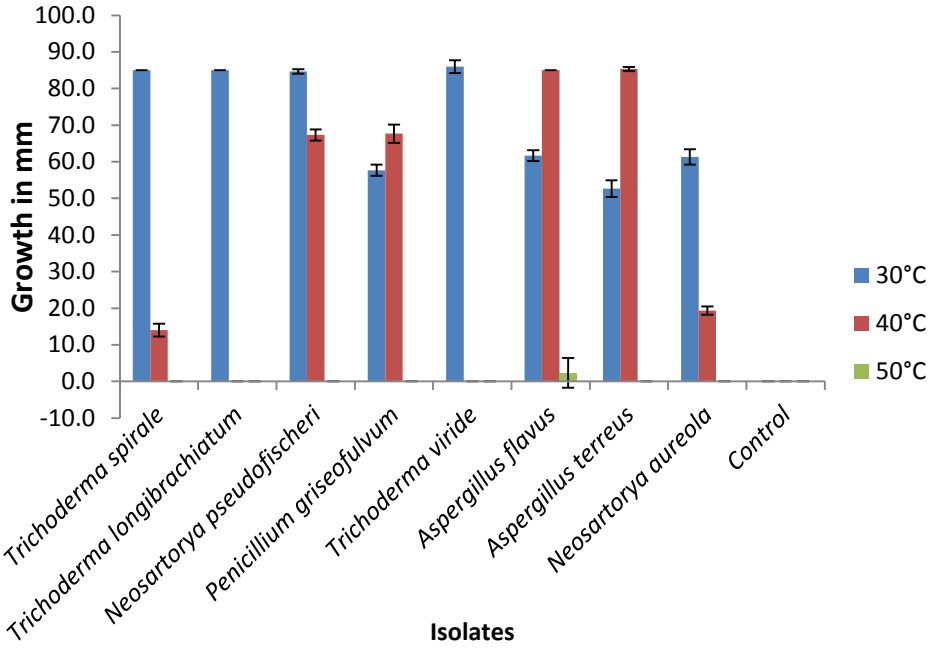

Figure 1: Effect of temperature on fungal growth

3.3 Fungal mycelial growth at different pH

There was optimal growth at pH 5, 7 and 9 by *Trichoderma spirale*, *Trichderma longibrachiatum*, *Neosartorya pseudofischeri* and *Aspergillus terreus.* Minimal growth was noted by *Penicillium griseofulvum*, *Trichoderma viride* and *Aspergillus flavus* at pH 5, 7 and 9. Poor radial growth was noted by *Neosartorya aureola* at pH 5, 7 and 9. However, the best growth was at pH 9 while poor growth was at pH 5. There was significance difference $p<0.05$ (Supplementary Table 2) on radial growth for fungal isolates at three different pH (Fig. 2).

4.0 DISCUSSION

From morphological characterization done using keys by Bamett and Hunter 1972, eight isolates namely; *Trichoderma viride*, *Trichoderma spirale*, *Neosartorya pseudofischeri*, *Neosartorya aureola*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium griseofulvum* and *Trichoderma longibrachiatum* were recovered from oil contaminated soils . The presence of these fungi in soil samples indicated that, the isolates were able to exist in the oil contaminated environment while those that could not survive in this environment being eliminated by the unfavorable conditions caused by the oil (Adekunke and Adebambo, 2007).

There was significant difference observed in the radial growth of the two fungal isolates under different temperatures. This indicates that, higher temperatures increase the rate of hydrocarbon metabolism to an optimal radial growth at 30°C and 40°C for the two isolates. Venosa and Zhu (2003) reported that, temperature plays very important roles in biodegradation of petroleum hydrocarbons, by its direct effect on the chemistry of the pollutants, effect on the physiology and diversity of the microbial communities. Highest degradation rates generally occur in the range of 30°C to 40°C in soil environments, 20°C to 30°C in some freshwater environments, and 15°C to 20°C in marine environments (Bossert and Bartha 1984). The radial

growth for the two isolates under different pH in this study was significantly different ranging from neutral to alkaline. The results of the present study is partly inconsistent with those obtained by Atlas (1988) who reported that heterotrophic bacteria favours a pH near neutrality, with fungi being more tolerant to acidic conditions.

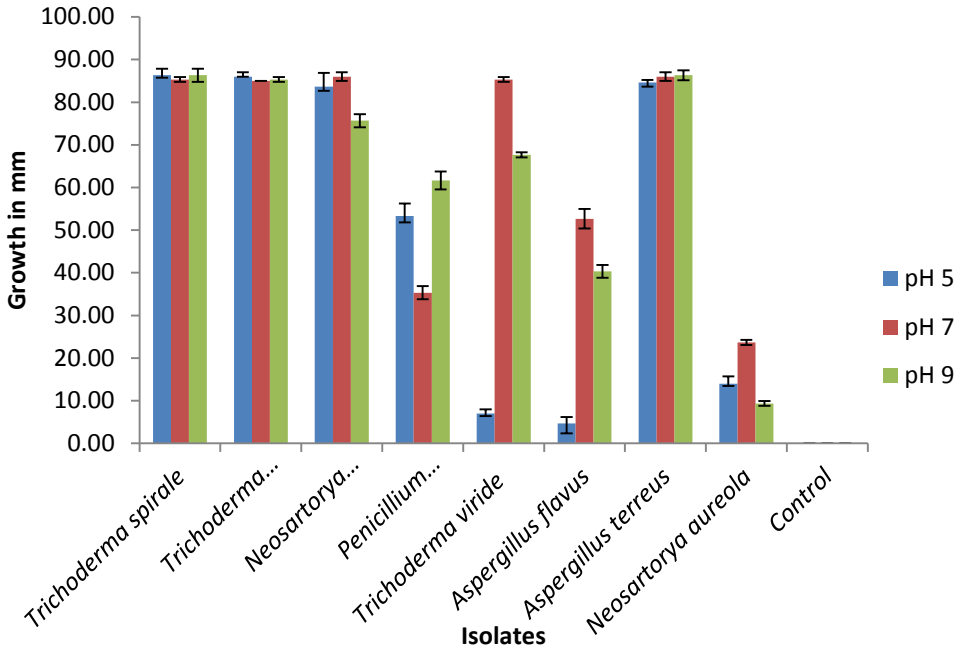

Figure 2: Effect of pH on fungal growth

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