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Assessment of Total Petroleum Hydrocarbon Degradation by Endophytic Fungi Isolated from Water Hyacinth *(Eichhornia Crassipes).*

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Abstract: Petroleum exploitation has negative impacts on the environment, such as oil spills, likewise, health implications on humans. The ability of newly isolated six fungal endophytes for degrading total petroleum hydrocarbons was evaluated. Their ability for hydrocarbon biodegradation was assessed in vitro for 35 days, using the minimal salt medium incorporated with products of crude oil, like petrol, kerosene, diesel, spent engine oil, unspent engine oil as the carbon source. Each fungus was introduced into test tubes containing minimal salt medium and petroleum products, the tubes were then incubated on shaker incubator at 27ºC, 151rpm for 35days. At every 7days, turbidity was measured with photoelectric colorimeter at wavelength of 530nm. In this study, the petroleum hydrocarbon degrading fungi isolated from Water Hyacinth leaves were identified using morphological techniques. The six fungi isolated were identified as; *Aspergillus niger, Penicillium canescens, Penicillium atrovenetum, Trichocladium opacum, Thermomyces lanuginosus* and *Penicillium* spp. In this experiment, it was evident that all the fungi were able to utilize hydrocarbons for their growth, at different rates. *Aspergillus niger* demonstrated high capacity to biodegrade diesel and spent engine oil, *Penicillium canescens* was excellent to utilize petrol, while *Penicillium atrovenetum* had the potential to degrade unspent engine oil and kerosene. The results obtained suggest that these endophytic fungal isolates are potential hydrocarbon biodegraders that could be used in bioremediation processes.

Keywords: TPH (Total petroleum hydrocarbon), endophytic fungi, degradation, water hyacinth, turbidity

I. Introduction

Water hyacinth *(Eichhornia crassipes)* is a free floating aquatic weed that is characterized by a rapid growth rate and it colonizes large water areas causing different problems [1]. Endophyte refers to all microorganisms that colonize internal plant tissues and their communities are either partially or completely responsible for the biosynthesis of host plant secondary metabolites [2]. All hydrocarbons compounds derived from petroleum sources are generally described as Total petroleum hydrocarbons (TPHs), and are categorized as aromatic, aliphatic, resin-based and asphaltene-based hydrocarbon [3]. The atmosphere serves as a vehicle for transportation and deposition of both TPHs and PAHs [4,5] and the aromatic hydrocarbon in the atmosphere adhere mainly to the atmospheric particulate matter. The environmental PAHs have their origin from anthropogenic activities like the release of incomplete combustion of organic materials (e.g., fossil fuels, wood and straw), vehicular emissions and many industrial processes. The street dust serves as a linkage of PAHs from the

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atmosphere to various surfaces. Thus, street dust has been considered an important vehicle for PAHs [6]. Intake of PAHs, especially particle-bound PAHs and TPHs can cause various cancer risks to humans, such as lung and skin cancer [7,8]. Therefore, evaluating the carcinogenic risks associated with PAHs in particulate matter is necessary for human health research.

As a result of the rapid technological advancement of industrialization leading to urbanization, the problem of environmental pollution caused by Polycyclic Aromatic Hydrocarbons (PAHs), black carbon (BC) and heavy metals becomes a serious concern to human health and the ecological environment [9,10,11]. Street dust is a means of assessing environmental quality in an urban area, and it was used to evaluate the PAHs, carbon components and heavy metals in street dust of industrial cities [12,13,14].

Microorganisms have been reported as principal agents in the degradation of petroleum hydrocarbons (PHs), with variations in the degradation efficiencies [15]. Also, it is generally suggested previously by different authors that microbes such as bacteria and fungi have the metabolic ability to use hydrocarbons as a carbon source [16]. This study is aimed to isolate endophytic fungi from water hyacinth capable of degrading petroleum hydrocarbon.

II. Materials and Methods

A. Sample collection

Plant sample (water hyacinth) was collected from river Osun in Osogbo town, Osun State, Nigeria under aseptic conditions and packed into sterile polythene containers and placed in a sterile polythene bag. It was then transported to the microbiology laboratory, Osun State University, Osogbo for processing within 24 hours of collection to prevent deterioration.

B. Identification of Fungal Isolates

The cultural and morphological characteristics of the isolates were observed and noted and formed part of the criteria to be used for identification. Morphological characterization of fungi isolates was done based on fungal appearance both surface and reverse, texture, and colour of the colony both on the surface and reverse. Detailed morphological characteristics of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single; the type of spore, etc were observed and recorded. Slides of pure cultures of the isolates were prepared for microscopic observation and identification by adding a drop of lacto phenol on to each slide and the pure isolate was picked onto the slide aseptically, it was then covered with a cover slip and viewed under the microscope with x40 objective. Fungal structure was described and photographed.

C. Isolation of Endophytic Fungi from the Plant Samples

The plant sample was washed thoroughly with distilled water to remove dirt and the leaves were then separated from the stems and the root. The leaves were cut into 2mm x 2mm pieces aseptically using a sterile surgical blade. The pieced leaves were surfaced sterilized by putting them in a beaker containing 100% ethanol for 1 minute, this was carried out three times and then the sterile leaves were immersed into another beaker containing distilled water to rinse excess ethanol using sterile forceps, the leaves were taken out of distilled water and placed on sterile filter paper to surface dry under aseptic condition, randomly selected pieces from the sample were planted into petri-dishes containing the media Potato Dextrose Agar (PDA). The Petri dishes were then incubated at room temperature (27°C) for 5 to 7 days. This protocol was done according to [17] with little modification.

D. Assessment of Hydrocarbon Utilization Potential of the Isolated Fungi

The enrichment procedure as described by [18] with little modifications was employed in the assessment of hydrocarbon utilization of isolated endophytic fungi. A minimal salt broth containing 2.0 g of NA_2HPO4, 0.17 g of K_2SO4, 4.0 g of NH_4NO3, 0.53 g of KH_2PO4, 0.10 g of MgSO4, 7H2O were dissolved in 1000 ml of distilled water. Ten millilitres of minimal salt broth medium was dispensed into 66 sterile test tubes and it was sterilized using autoclave at 121°C for 15minutes. 2ml of kerosene, diesel, unspent engine oil, spent engine oil, petrol was dispensed to 10 ml of minimal salt broth in the test-tube rack, while the last rack was prepared without the fungi to serve as the control.

By observing the aseptic procedure, six fungi isolated from water hyacinth were inoculated into each test tube containing hydrocarbon except for their control tubes, each of the test tubes was plugged with sterile cotton wool wrapped with aluminium foil so as to ensure maximum aeration and prevent cross – contamination. The tubes were arranged into test- tube rack. The rack was then placed inside a shaker incubator of (28 - 31°C) at 151 rpm. The experiment was placed in a shaker incubator to facilitate oil cell phase layer. The growth rate of fungi in MSB was measured at every 7 days using a photoelectric colorimeter at a wavelength of 530 nm for 36days.

III. Results and Discussion

The results of morphological characteristics of isolated fungi are presented in table 1

A. Growth Rate of Endophytic Fungi in Minimal Salt broth Mixed with Petroleum Hydrocarbons (PHs)

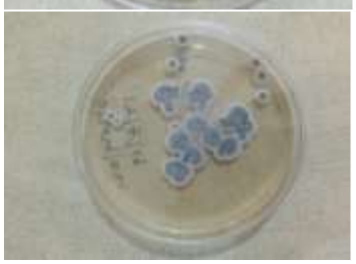
The growth pattern of the endophytic fungi varies in the utilization of total petroleum hydrocarbons as the growth rates of each isolate were measured every 7days with photoelectric colorimeter at the absorbance of 530nm. In petrol, PNF 06 had the highest growth rate at 35^{th}day with a turbidity of 0.58 followed by PNF 01 with a turbidity of 0.51 while PNF 05 with the lowest turbidity of 0.33 to utilize petrol as shown in fig. 1.

It was observed in fig. 2 that PNF 03 had the highest growth rates and the ability to degrade diesel with a turbidity of 0.81 at the 35^{th} day followed by PNF 02 and PNF 05 with the turbidity of 0.55 while PNF 06 with the lowest turbidity of 0.28.

In kerosene, PNF 01 was observed to be the best to degrade kerosene at the 35^{th} day with the turbidity of 0.77 followed by PNF 03 with turbidity of 0.58 while PNF 05 with the lowest turbidity of 0.07 as shown in fig. 3. It was observed in spent engine oil that PNF 03 had the highest growth rate with turbidity of 0.64 and PNF 01 with turbidity of 0.35 at 35^{th} day while PNF 06 had the lowest ability to utilize spent engine oil with turbidity of 0.18 as presented in fig. 4.

PNF 01 had the highest growth rates in unspent engine oil with a turbidity of 0.47 and PNF 06 with the turbidity of 0.17 while PNF 05 had the lowest growth rate with the turbidity of 0.05 at 35^{th} day to utilize unspent engine oil as shown in fig. 5.

Table 1: Morphological Characteristics of Fungi Isolates

Isolate code	Growth	Surface	Microscopic view x40	Presumptive Identification
PNF 01	bluish white surface			*Penicillium atrovenetum*
PNF 02	Greenish surface with pink vinaceous			*Thermomyces lanuginosus*
PNF 03	Black surface with powdery texture			*Aspergillus niger*
PNF 04	White surface, wooly texture			*Penicillium spp*
PNF 05	Greenish with powdery surface			*Trichocladium opacum*
PNF 06	Bluish surface			*Penicillium canescens*

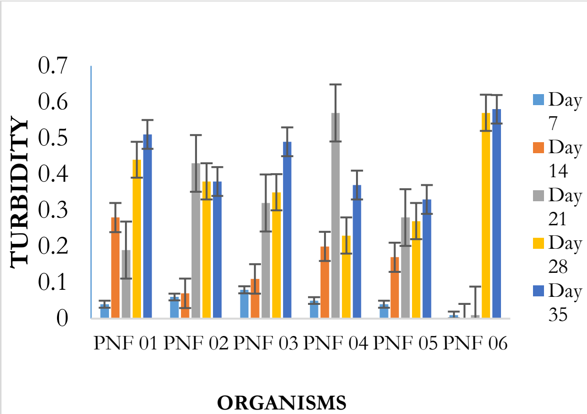


Figure. 1: The Growth rate of Fungi in Petrol and Minimal Salt Broth Medium (MSB).

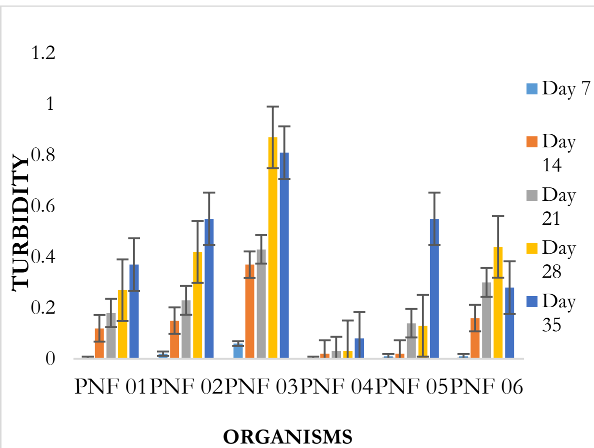


Figure. 2: The Growth rate of Fungi in Diesel and Minimal Salt Broth Medium (MSB).

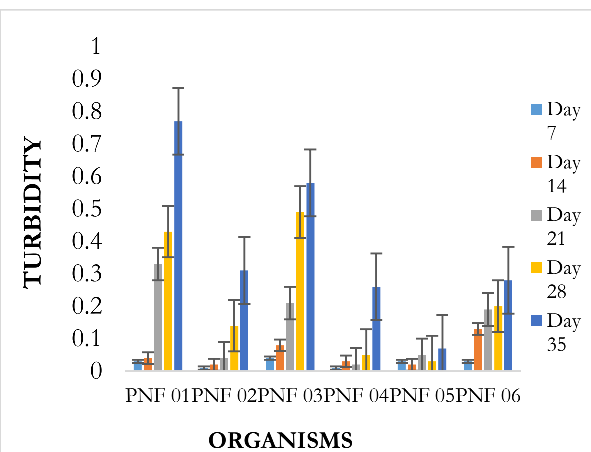


Fig. 3: The Growth rate of Fungi in Kerosene and Minimal Salt Broth Medium (MSB).

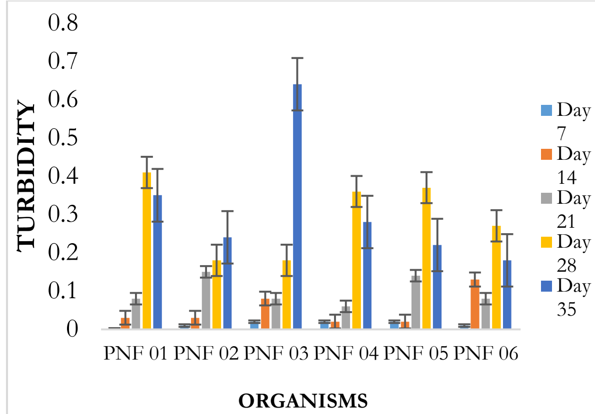


Figure. 4: The Growth rate of Fungi in Spent Engine oil and Minimal Salt Broth Medium (MSB)

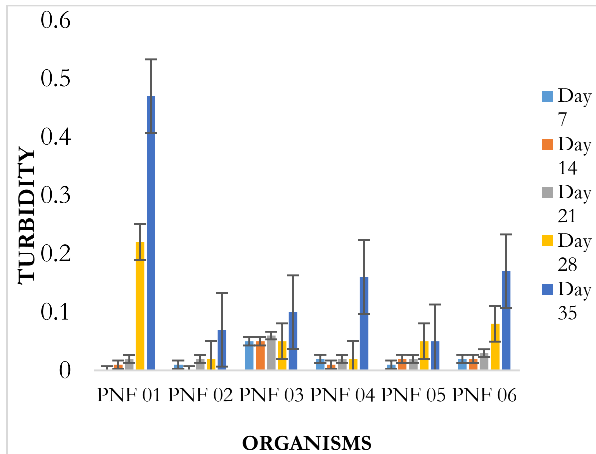


Figure. 5: The Growth rate of Fungi in Unspent Engine oil and Minimal Salt Broth Medium (MSB)

KEY

PNF 01	*Penicillium atrovenetum*
PNF 02	*Thermomyces lanuginosus*
PNF 03	*Aspergillus niger*
PNF 04	*Penicillium spp*
PNF 05	*Trichocladium opacum*
PNF 06	*Penicillium canescens*

B. Discussion

The presence of Total Petroleum Hydrocarbons (TPHs), and heavy metals in the environment has contributed immensely to the problem of environmental pollution, thereby posing serious challenges to human and animal health. Therefore, the use of microorganisms to bioremediate contaminated environments has been an alternative means to the use of chemicals as a remediation method which may result in a negative effect on human health. In this bioremediation study carried out in the laboratory, six fungi were isolated from water hyacinth, and were identified as; *Penicillium atrovenetum, Thermomyces lanuginosus, Penicillium canescens, Trichocladium opacum, Aspergillus niger* and *penicillium spp*. All the fungi isolated were observed to have increased growth rates in minimal salt broth medium (MSB) containing TPHs. It could be suggested that the isolates were able to use the hydrocarbons in the (MSB) as a source of energy, by releasing enzymes that are capable of breaking down hydrocarbons to simpler forms or to products that serve as their nutrients [19].

In this study, *Aspergillus niger* demonstrated active ability to utilize diesel and spent engine oil which is in line with a report of [20] that *Aspergillus niger,* and *Penicillium spp* have active potential to degrade PAHs. Also, according to [21] fungi isolated from the aquatic environment had the capacity to utilize petroleum hydrocarbons. Furthermore, *Penicillium canescens* was excellent to utilize petrol, *Penicillium atrovenetum* had the capacity to degrade unspent engine oil and kerosene as reported also by [19] that they were capable of degrading hydrocarbons. [22] reported that an increase in the growth rates of the fungal isolates might be traced to the fact that the isolates utilize hydrocarbons as a substrate for their growth by using extracellular enzymes to breakdown the recalcitrant hydrogen molecules, dismantling the long chains of hydrogen and carbon and then convert petroleum to simpler products.

IV. Conclusion

From this study, six endophytic fungi were isolated from water hyacinth, namely; *Penicillium atrovenetum, Thermomyces lanuginosus, Penicillium canescens, Trichocladium opacum, Aspergillus niger* and *penicillium spp* and were observed to have the potential to utilize TPHs as their source of carbon and also for their growths. In conclusion, more research is required to study the mechanisms of degradation and in situ bioremediation of the fungal isolates.

Acknowledgement

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Conflict of Interest

This work does not have any conflict of interest.

References

[1] Zhang, Y., Zhang, D. and Barrett, S. "Genetic Uniformity Characterizes the Invasive Spread of Water Hyacinth *(Eichhornia Crassipes*), a Clonal Aquatic Plant", *Molecular Ecology,* vol. 19, 2010, pp. 1774-1786.

[2] Ludway-muller, J. "Plant and Endophytes: Equal Partners in Secondary Metabolites Production", *Biotechnology Leterst*, vol. 37, 2015, pp. 1325-1333.

[3] Tisot, B. and Welte, D. "Biological Productivity of Modern Aquatic Environments", *Springer Science and Business Media*, New York, 2013.

[4] Lee, B.K. and Dong, T.T. "Toxicity and Source Assignment of Polycyclic Aromatic Hydrocarbons in Road Dust from Urban Residential and Industrial Areas in a Typical Industrial City in Korea", *Journal of Material Cycles Waste Management,* vol. 13, 2011, pp. 34–42.

[5] Keyte, I.J., Harrison, R.M. and Lammel, G. "Chemical Reactivity and Long-range Transport Potential of Polycyclic Aromatic Hydrocarbons–A

Review", *Chemical Society Review,* vol. 42, 2013, pp. 9333–9391.

[6] Wu, Y., Luo, Y., Zou, D., Ni, J., Liu, W., Teng, Y. and Li, Z. "Bioremediation of Polycyclic Aromatic Hydrocarbons Contaminated Soil with *Monilinia* sp.: Degradation and Microbial Community Analysis", *Biodegradation*, vol. 19, no. 2, 2008, pp. 247–257.

[7] Kim, K.H., Jahan, S.A., Kabir, E. and Brown, R.J. "A Review of Airborne Polycyclic Aromatic Hydrocarbons (PAHs) and their Human Health Effects", *Environment International,* vol. 60, 2013, pp. 71–80.

[8] Yue, H., Yun, Y., Gao, R., Li, G. and Sang, N. "Winter Polycyclic Aromatic Hydrocarbon-bound Particulate Matter from Peri-urban North China Promotes Lung Cancer Cell Metastasis", *Environmental. Science and Technology,* vol. 49, 2015, pp. 14484–14493.

[9] Han, Y., Wei, C., Bandowe, B.A., Wilcke, W., Cao, J., Xu, B., Gao, S., Tie, X., Li, G. and Jin, Z. "Elemental Carbon and Polycyclic Aromatic Compounds in a 150- year Sediment Core from Lake Qinghai, Tibetan Plateau, China: Influence of Regional and Local Sources and Transport Pathways", *Environmental Science and technology*, vol. 49, 2015, pp. 4176–4183.

[10] Wei, C., Han, Y., Bandowe, B.A.M., Cao, J., Huang, R.J., Ni, H., Tian, J. and Wilcke, W. "Occurrence, Gas/particle Partitioning and Carcinogenic Risk of Polycyclic Aromatic Hydrocarbons and their Oxygen and Nitrogen Containing Derivatives in Xi'an, Central China, *Science of the Total Environmenta,* vol. 505, 2015, pp. 814–822.

[11] Zhang, Y., Zhang, D. and Barrett, S. "Genetic Uniformity Characterizes the Invasive Spread of Water Hyacinth *(Eichhornia crassipes),* a Clonal Aquatic Plant", *Molecular Ecology,* vol. 19, 2010, pp. 1774-1786.

[12] Amato, F., Pandolfi, M., Viana, M., Querol, X., Alastuey, A. and Moreno, T. "Spatial and Chemical Patterns of PM10 in Road Dust Deposited in Urban Environment", *Atmospheric Environment,* vol. 43, no. 9, 2009, pp. 1650–1659.

[13] Lu, X., Wang, L., Li, L.Y., Lei, K., Huang, L. and Kang, D. "Multivariate Statistical Analysis of Heavy Metals in Street Dust of Baoji, NW China", *Journal of Hazardous Material,* vol. 173, 2010, pp. 744–749.

[14] Leitao, A.L. and Enguita, F.J. "Gibberellins in *Penicillium* Strains: Challenges for Endophyte-plant Host Interactions Under Salinity Stress", *Microbiological Research,* vol. 183, 2016, pp. 8–18.

[15] Khan, R., Shahzad, S. and Choudhary, M. "Communities of Endophytic Fungi in Medicinal Plant Withania Somnifera", *Pakistan Journal of Botany*, vol. 42, 2010, pp. 1281-1287.

[16] Hammel, K.E. "Mechanisms for Polycyclic Aromatic Hydrocarbons. Degradation by Ligninolytic Fungi", *Environmental Health Perspectives*, vol. 103, 1995, pp. 41.

[17] Strobel, G.A., Dirkse, E., Sears, J. and Markworth, C. "Volatile Antimicrobials from Muscodor albus, a Novel Endophytic Fungus. Microbiology", vol. 147, 2001, 2943-2950. https://doi.org/10.1099/00221287-147-11.

[18] Nwachukwu, S.C.W. "Enhanced Rehabilitation of Tropical Aquatic Environment Polluted with Crude Petroleum using *Candida Utilis", Journal of environmental biology*, vol. 21, no. 3, 2000, pp. 241-250.

[19] Okerentugba, P.O. and Ezeronye, O.U. "Petroleum Degrading Potentials of Single and Mixed Microbial Cultures Isolated from Rivers and Refinery Effluent in Nigeria", *African Journal of Biotechnology,* vol. 2, no. 9, 2003, pp. 288 – 292.

[20] Gesinde, A.F., Agbo, E.B., Agho, M.O. and Dike, E.F.C. "Bioremediation of Some Nigerian and Arabian Crude Oils by Fungal Isolates", *Int Jor P App Scs,* vol. 2, 2008, pp. 37-44.

[21] Bartha, R. and Atlas, R.M. "The Microbiology of Aquatic Oil Spills", *Advance Applied Microbiology,* vol. 22, 1997, pp. 225-266.

[22] Adekunle, A.A. and Adebambo, O.A. "Petroleum Hydrocarbon Utilization by Fungi Isolated from Detarium Senegalense (J. F Gmelin) Seeds", *Journal of American Science,* vol. 3, 2007, pp. 69-76.