The Abilities of Four Species of Nigerian Aquatic Phycomycetes to Utilize Petroleum and Petroleum Products as Sole Carbon Sources

Odu, C.E., MSc
Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria
Ogbonna, C.I.C., PhD
Applied Microbiology and Biotechnology Research Unit, Dept of Plant Science and Technology,University of Jos, Jos, Nigeria
Bukbuk, D., PhD
Department of Microbiology, University of Maiduguri, Maiduguri, Nigeria
Chukwu, O.O.C., PhD
Molecular Biology Department, FCVMLT, VOM, Jos, Nigeria
Yerima, I., MSc
Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria
Mustapha, D., BSc
Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria

Doi: 10.19044/esj.2018.v14n3p240 URL:http://dx.doi.org/10.19044/esj.2018.v14n3p240

Abstract

Studies were carried out on the abilities of four aquatic phycomycetes species isolated from crude oil polluted marine , brackish and fresh water environments in Nigeria to utilize refined Petroleum and Petroleum products as sole sources of carbon. The said species of fungi included *Brevilegnia indica*, *Protoachyla paradoxa*, *Saprolegnia bhargavi* and *Thraustotheca clavata*. They were grown on fungal culture media which contained mineral salts solution, refined petroleum, kerosene and diesel as sole sources of carbon and agar as a solidifying agent. Two concentrations of each of the resulting oil agar media were used vis 1% and 2%. The resultant medium was then used to culture each of the test fungi in triplicates. The resultant culture plates were then incubated at 25 ^{O}C and left for daily observation. The test fungi that grew on each medium were observed for their abilities to emulsify the refined petroleum or its products (diesel and kerosene). Control experiments were also set up using Malt Extract Agar medium. *Brevilegnia indica* grew on 1%

petroleum and diesel growth media but did not grow on kerosene medium. *Protoachyla paradoxa* grew minimally on diesel medium at 1% and 2% compositions. *Saprolegnia bhargavi* did not grow on the petroleum agar medium or petroleum products media. *Thraustotheca clavata* grew minimally on the kerosene medium at both 1% and 2% compositions. The four test fungi emulsified the diesel agar medium at both 1% and 2% compositions. *Brevilegnia indica* emulsified only petrol at 1% while Non of the isolates emulsified the kerosene medium at both 1% and 2% compositions. Oil globules were also observed in the vegetative hyphae of the test fungi which grew on the experimental culture media. This means that such fungi either degraded or accumulated the petrol or petroleum products in their systems.

Keywords: Aquatic phycomycetes, Petroleum, Diesel, Kerosene, oil agar media, Polluted environment

Introduction

Petroleum and petroleum products forms one of the major pollutants of the aquatic environment especially in the Niger-Delta region of Nigeria and in freshwater bodies used for industrial and domestic purposes like washing of petrol and diesel engines and automobiles. Oil spillage is the accidental discharge or pouring of crude oil into the environment. It involves the contamination of any part of the environment with hydrocarbon. These spills endanger public health, imperil drinking water, devastate natural resources and disrupt the economy (Gesinde *et al*., (2008)). Crude oil is a naturally occurring complex mixture of hydrocarbon and non hydrocarbon compounds which at appropriate concentration possesses a measurable toxicity towards living systems. The toxicity of crude oil or petroleum products vary widely depending on their composition, concentration, environmental factors and on the biological state of the organisms at the time of the contamination Obire and Anyanwu (2009). Although oil spills contaminates living and nonliving, microorganisms especially fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the environment and have mechanisms for the elimination of spilled oil from the environment (Dibble and Bartha (1979), Atlas (1995)). Aquatic phycomycetes are the group of primitive fungi that have adapted to the aquatic environment by the possession of flagella for motility. Aquatic phycomycetes are broadly divided into two groups based on possession of one or two flagella as Chytridiomycetes and Oomycetes respectively (Khulbe, 2001). These fungi contribute to the energy flow and productivity of aquatic and semi aquatic ecosystem by their active role in the utilization and bio deterioration of a variety of organic and inorganic materials (Khulbe, 2001). In the aquatic ecosystem, fungi therefore play important role with their ability in removing

hazardous compounds from water, thus fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria (Westlake *et al.*,1974).

Recently many researchers studied the role of fungi in biodegradation process of petroleum products and the most common fungi which have been recorded as biodegraders belongs to the following genera: *Alternaria, Aspergillus, Candida, Cephalosporium, Fusarium, Geotrichum, Gliocladium, Mucor, Paecilomyces, Penicillium, Pleurotus, Polyporus, Rhizopus, Rhodotolura, Saccharomyces, Talaromyces* and *Torulopsis* (Gesinde *et al*., (2008), Obire and Anyanwu (2009), Adekunle and Adebambo (2007), Hadibarata and Tachibana (2009), Adekunle *et al*., (2004), Atagana *et al*., (2006), Hussein *et al.*, (2008), Romero *et al.*, (2010), Saraswathy and Hallberg (2002). Reviews on Fungi in bioremediation of crude oil across the world (Nilanjana and Preethy (2011), Jahangeer and Vikram (2013)) and in Nigeria (Obire and Putheti (2009) recorded yeasts and filamentous fungi as fungi biodegraders of crude oil. None of the reviewers encountered in this study reported aquatic phycomycetes as biodegraders of crude oil. However, Yerima *et al.* (2009) carried out a laboratory based degradation of light crude oil by aquatic fungi suspected to be aquatic phycomycetes isolated in River Sokoto, a non oil producing region and got results that seemed to indicate that aquatic phycomycetes may have a potential use in bioremediation. In view of the fact that most research work done on biodegradation of crude oil and its fractions have centered on yeasts and filamentous fungi, this research work is therefore an attempt to test the abilities of aquatic phycomycetes to degrade crude oil and its fractions especially in some water bodies in Jos, Plateau State and the oil rich Bayelsa State of Nigeria. This study is more important because most of the studies on biodegradation of crude oil or its fractions have not centered on aquatic phycomycetes but on other aquatic fungi and have not been carried out in the oil rich Niger-Delta area and Jos areas of Nigeria. Hence this study may be one of the pioneering preliminary works assessing possibility of using aquatic phycomycetes as a biodegradation agent.

Materials and methods

Sampling sites and sampling procedures: The sampling sites where water samples for the isolation of aquatic phycomycetes for the study were collected included three sampling points on River Nuns in Odi LGA, Nembe seaport, Ogbia waterside in Bayelsa state and three freshwater bodies in Jos, Plateau State namely Rayfield resort lake, Dorowa pond by school of health, Zawan and Zawan pond by Zawan barricade. Water samples for Plateau State were collected in February 2013 while those from Bayelsa were collected in the first week of March 2013. Water samples were collected with the aid of sterile 500mls bottles aseptically and transported to the Dermatophilosis laboratory

of National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State in coolers designed as hand refrigerator with icepacks maintained at 5^{o}C. For the samples collected from Bayelsa State, they were equally stored in coolers designed as hand refrigerator with icepacks maintained at 5^{o}C and freighted to the laboratory in Jos within 24hrs. Analyses of samples were carried out as soon as practicable on the day of collection but not more than 24hours after collection especially water samples from Bayelsa State (Marano *et al*., 2008; Branislav, 2005).

Isolation of aquatic phycomycetes for culture based studies

The isolation of aquatic phycomycetes was carried out during the dry season of February and March 2013 using hemp seed (*Canabis sativum*) and sesame seed (*sesamum indicum*) as baits according to the methods of Alabi (1971) and Ogbonna and Alabi (1991). In this method, Hemp seed (*Canabis sativa*) and Sesame seed (*Sesamum indicum*) were separately collected and boiled. The boiled seeds were then washed in 5 changes of sterile distilled water. The washed seeds were then divided into batches and used to plate out the water samples (5seeds) per plate of water sample. In order to suppress bacteria contaminants, 4 drops of streptomycin sulphate solution were added to each plate of water sample. The resultant plates containing water samples and inoculated seeds were incubated at 25^{o}C (room temperature) and observed daily for growth of aquatic phycomycetes (Marano *et al*., 2008, Nascimentol *et al*., 2011 and Trifa and Adiba, 2011). The colonies appearing on baited seeds were separated based on closely observed morphological differences like colour, length of hyphae, concentration of hyphae around the seed and in conclusion differentiation into species was based on morphological differences of zoosporangia and hyphae. Baited seeds with aquatic phycomycetes isolates showing similar morphological characteristics were pulled together into Petri-dishes containing sterile distilled water to which antibiotics had been added to suppress bacteria growth and observed directly under the compound light microscope for identification according Trifa and Adiba (2011), Marano *et al.* (2008), Nascimentol *et al.* (2011), El-Hissy *et al.* (2000) and Branislav (2005). Results as seen from the microscope under appropriate magnifications were then compared for similarity with pictures and descriptions found in manual for identification of aquatic fungi (Khulbe 2001) and aquatic phycomycetes (Sparrow 1960).

Rebaiting of baited aquatic phycomycetes

Four species of aquatic phycomycetes showing consistency in occurrence and presenting with clear identification properties both morphologically and microscopically were selected for the culture based studies on solid culture media. The selected species were *Brevilegnia indica,*

Protoachyla paradoxa, Saprolegnia bhargavi and Thraustotheca clavata. However to get sufficient hyphae for the culture based studies, each of the fully identified isolates on colonized baits were washed in sterile distilled water and transferred to fresh Petri-dishes containing distilled water to which an antibiotics (streptomycin sulphate) had been added to prevent bacteria growth. Fresh boiled seeds (new baits) of either hemp or sesame were then introduced aseptically into the Petri-dishes containing the purified isolates of aquatic phycomycetes. The resulting Petri-plates were then incubated at room temperature and observed for presence of similar aquatic phycomycetes on the new baits after three to four days and confirmed by morphological and direct microscopic observation. The new baits were observed to have trapped similar isolates of aquatic phycomycetes in each of the Petri-dishes seeded. These way the aquatic phycomycetes isolates were concentrated to have more hyphae for the culture based studies.

The abilities of four species of Nigerian aquatic phycomycetes to utilize petroleum and petroleum products as sole carbon sources.

Studies were carried out on the abilities of four species of aquatic phycomycetes isolated from crude oil polluted marine, brackish and freshwater environments in Nigeria to utilize refined petroleum and petroleum products as sole carbon sources. The said species of aquatic phycomycetes included *Brevilegnia indica, Protoachyla paradoxa, Saprolegnia bhargavi and Thraustotheca clavata*. The aquatic phycomycetes were grown in mineral salt media prepared according to the pioneering method of Zajic and Supplisson (1972) with the following composition: K_2HPO_4=1.8g, NH_4CL=4.0g, $MgSO_4.7H_2O$=0.01g, Nacl=0.1g, Agar=20g and made up with 1000mls of distilled water. Drops of streptomycin sulphate (0.3g in 1000mls of distilled water) were added to suppress bacteria growth.

6 Sets of 300mls of the above mineral salt media with antibiotics to suppress bacteria growth were poured into 500mls autoclavable bottles and conical flasks and sterilized by autoclaving at 121^0C for 15minutes.

The sole carbon sources, petroleum, diesel and kerosene were filtered with Millipore filter core paper (AP 2029300) made in Bedford Massachusettes, USA.

Two sets of the 300mls MSM media were separated for each of the three filtered sole carbon sources. In each group one set was used to prepare media with 1% v/v supplement while the second had 2% v/v supplement.

The filtered petroleum and petroleum products (diesel and kerosene) were then added separately in 1%v/v and 2% v/v into separate sets of the sterilized Mineral salt media as follows: 1%v/v means 3mls into 300mls MSM while 2%v/v means 6mls into 300mls MSM.

The resulting oil mineral salt media (1%v/v petroleum MSM, 2%v/v petroleum MSM, 1%v/v diesel MSM, 2%v/v diesel MSM, 1%v/v kerosineMSM and 2%v/v kerosene MSM) were each poured in 20mls amounts into five Petridishes in triplicates and allowed to set/solidify up to 45^{o}C.

Four of the five plates were each inoculated centrally with each of the four test isolate of aquatic phycomycetes earlier enumerated. The fifth plate was not inoculated with any test organism and served as control.

Malt extract agar medium was also prepared alongside according to manufacturer's instruction and used to culture the four test aquatic fungi following the procedure above for oil Mineral Salt Medium.

All the plates inoculated with test aquatic fungi and the controls without test organisms were incubated at room temperature and observed daily between 1-7 days for growth and emulsification properties according to Zajic and Supplisson (1972).

Result

The test aquatic phycomycetes , *Thraustotheca clavata* was isolated from River Nuns at Nembe seaport and Ogbia waterside while *Saprolegnia bhargavi* was isolated from River Nuns in Odi LGA of Bayelsa State. *Protoachyla paradoxa* was isolated from Rayfield resort lake, Jos while *Brevilegnia indica* was isolated from Dorowa pond by School of Health in Zawan and Zawan pond by Zawan barricade. The test fungi that grew on each Mineral salt medium oil agar were observed for their abilities to emulsify the refined petroleum or its products (diesel and kerosene). The extent of growth and emulsification effect on media surface is presented in Table1. Table 1 above was deduced from the experimental set up as shown in Plate 1. The following can be deduced from Table 1: *Brevilegnia indica* grew on 1 %(v/v) petroleum and diesel Mineral salt media(MSM) but did not grow on kerosene Mineral salt medium. *Protoachyla paradoxa* grew minimally on diesel medium at 1 %(v/v) and 2%(v/v) compositions as shown in Plate 2 while its growth on Malt extract agar medium is shown in Plate 3. *Saprolegnia bhargavi* did not grow on the petroleum mineral salt agar medium or petroleum products media but showed emulsification on 1% &2% diesel MSM as shown in Plate 4. The Plate 4 also shows the colonial morphology of *Saprolegnia bhargavi* on malt extract agar medium. *Thraustotheca clavata* grew minimally on the kerosene medium at both 1 %(v/v) and 2%(v/v) compositions as seen in Plate 5 for growth as black spores on 1% (v/v) kerosine and luxuriant growth on malt extract agar starting with the same black spores and white hyphae. Plate 6 shows slight emulsifation on 2% diesel MSM. The four test fungi emulsified the diesel agar medium at both 1 %(v/v) and 2%(v/v) compositions.

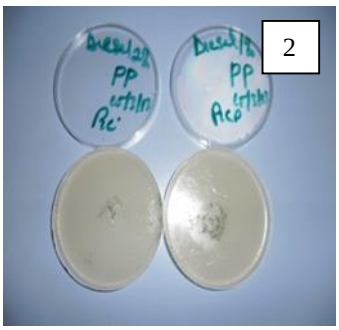
Brevilegnia indica emulsified only petrol at 1%(v/v) while Non of the isolates emulsified the kerosene medium at both 1%(v/v) and 2%(v/v) compositions.

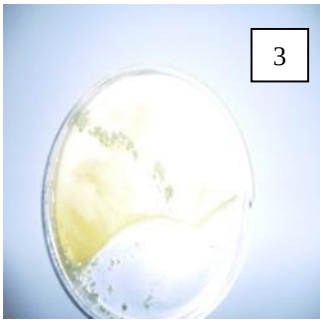
Table 1: Degree of emulsification and growth of aquatic phycomycetes on oil mineral salt media

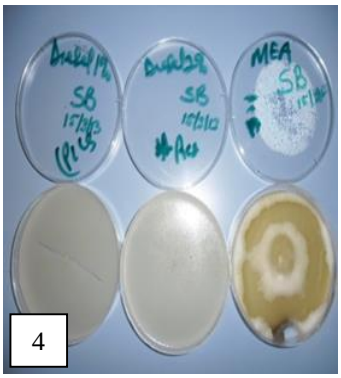
Test isolates		Kerosine MSM		Petrol MSM		Diesel MSM		MEA
		1%	2%	1%	2%	1%	2%	
Thrausthotheca clavata	Minimal growth	+	+	_	_	_	_	++++
	Emulsification	_	_	_	_	++	++	
Brevilegnia indica	Minimal growth	_	_	+	_	+	_	++++
	Emulsification	_	_	+	+	++	++	
Saprolegnia bhargavi	Minimal growth	_	_	_	_	_	_	++++
	Emulsification	_	_	_	_	++	++	
Protoachyla paradoxa	Minimal growth	_	_	_	_	+	+	++++
	Emulsification	_	_	_	_	++	++	
No fungal inoculation (control)	Growth	_	_	_	_	_	_	_
	Emulsificaton	_	_	_	_	_	_	

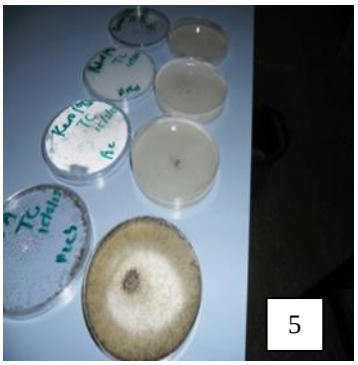
Note: MEA; Malt extract agar, MSM; Mineral salt medium, Test isolates; Aquatic Phycomycetes species

The four isolates showed good growth on malt extract agar medium while there were no growths as expected on the plates containing oil mineral salt media without inoculation of any of the isolates. Since there was no growth/emulsification on Petri plates without fungal inoculation, it means that the test organisms were responsible for the minimal growth and emulsification observed on the Petri- plates. Emulsification of supplemented petroleum and petroleum products and minimal growth observed in the experiment are indications of biodegradation potentials of these isolates.









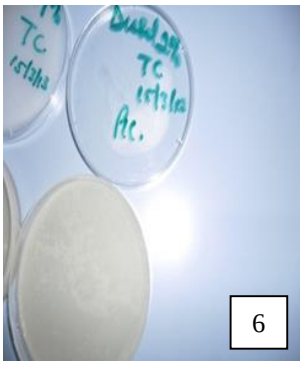


Plate 1: Shows experimental setup for ability of four aquatic phycomycetes to utilize petroleum and petroleum products

Plate 2: *Protoachyla paradoxa* on 1%&2% diesel MSM showing same tinge of green spores without hyphal growth but with slight emulsification

Plate 3: *Protoachyla paradoxa* on Malt extract agar showing white hyphae (as white as snow) with a tinge of green spores from centre of inoculation

Plate 4: *Saprolegnia bhargavi* shows hyphal growth on MEA on last Petri-dish and no growth on 1%&2% diesel MSM.

Plate 5: Shows *Thraustheca clavata*, first Petri-dish on malt extract agar showing over grown white hyphae with numerous discharged black spores while second petridish show same black spores at centre of inoculation as only growth on 1% kerosine MSM.

Plate 6: *Thraustheca clavata* showing only emulsification on 2% diesel MSM and no hypha or spore growth.

Discussion

The studies on the abilities of four Nigerian aquatic phycomycetes to utilize petrol and petroleum products were positive. The method of purifying isolates by rebaiting after changes in sterile distilled water was tested in this study since aquatic phycomycetes are aquatic in nature thus maintaining them in their natural environment should bring out the best of their abilities. Purification of isolates by rebaiting method was successful and it was observed that *Brevilegnia indica* grew on 1% petrol and diesel but not on kerosene supplemented MSM agar medium. *Protoachyla paradoxa* grew minimally at 1% and 2% on diesel supplemented MSM agar medium. *Thraustotheca clavata* also grew minimally at 1% and 2% kerosene supplemented MSM agar medium but there were no growth for *Saprolegnia bhargavi* on any of the petrol or petroleum products supplemented MSM agar media. Spectacular growth that were shown by *Protoachyla paradoxa* and *Thraustotheca clavata* on malt extract agar revealing luxuriant hyphal growth with tinge of green and black spores respectively were worthy of note. The two isolates showed minimal growths on supplemented media by producing same green and black spores respectively. This colored spores arose from the centre point of inoculation of parent hyphae and therefore arose from the

hyphae that may not have grown into mycellium. This may therefore mean survival reproductive structures of these isolates that probably accumulates the supplemented oil in form of oil globules since some aquatic phycomycetes's oosphere within oogonium contain oil globules whose positioning within the oosphere makes them centric or eccentric(Vashishta and Sinha, 2002). With regards to emulsification, all four test fungi emulsified 1% and 2% diesel MSM agar medium, *Brevilegnia indica* emulsified only 1% petrol MSM agar medium while none of the test fungi emulsified both 1% and 2% kerosene MSM agar medium.

To support the fact that the minimal growth observed and the emulsification evidence was as a result of the supplemented petrol and petroleum products was the observation that good mycelia growth were obtained on the conventional medium for fungal growth, Malt extract agar. In the same vein, the second control culture plates supplemented with petrol or petroleum products without inoculation of any of the test species of aquatic phycomycetes had no trace of mycelia or spore growth or any sign of emulsification or change on the surface of the media. There were also no growth on the malt extract agar media without inoculation of test aquatic phycomycetes species. Hence all the observed minimal growth and evidence of emulsification could only have come as a result of the supplements in the minimum salt medium agar medium. Since these minimal growths on the supplemented media came from the supplements , it then means that this preliminary study suggests that the four Nigerian aquatic phycomycetes had the abilities to some extent of utilizing these petrol and petroleum products as source of carbon for growth.

A closer look at the abilities of these four aquatic phycomycetes at using these supplements shows the highest preference for diesel since two out of the four species recorded minimal growth on it. Petrol and kerosene followed jointly since one each of the four species of aquatic phycomycetes grew minimally on them. Oil globules that were also observed in the vegetative hyphae of the test fungi on the experimental culture media also confirms the abilities of the test fungi to using the supplements as sole carbon source. This means that these fungi either degraded or accumulated the petrol or petroleum products.

In line with the results of this work, Dibble and Bartha (1979) and Atlas (1995) also concluded that microorganisms especially fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the environment and have mechanisms

for the elimination of spilled oil from the environment, According to Westlake *et al.*,(1974), microbes use petroleum hydrocarbon as nutrients in the aquatic ecosystem and also stated that the use of the same crude oil can favor different genera at different temperature. The oil polluted environment of

Niger-Delta region in Nigeria where some of the test species of aquatic phycomycetes were isolated have also been reported by Okerentugba and Ezeronye (2003) as source (particularly Rivers and refinery effluents) of bacteria and fungi species (*Aspergillus spp, Penicillium spp and Rhizopus spp*) that could degrade crude oil. It is important to note that reviews on fungi in bioremediation of crude oil across the world and in Nigeria have recorded Yeast and filamentous fungi as fungi biodegraders of crude oil. None of the reviewers have reported aquatic phycomycetes as biodegraders of petrol and petroleum products. However, Yerima *et al*., (2009) carried out a laboratory based degradation of light crude oil by a single species of aquatic phycomycetes which was not well highlighted and defined and got results that also in line with the result of this work seemed to indicate that aquatic phycomycetes have a potential use in bioremediation.

Conclusion

This preliminary study on abilities of aquatic phycomycetes to utilize petroleum and petroleum products on supplemented mineral salt agar medium was positive and confirmed that test isolates of aquatic phycomycetes from oil polluted environment could indeed utilize petroleum and petroleum products as sole carbon source and therefore could be further assessed for their greater role in bioremediation of oil polluted environment. Since most studies on biodegradation of oil pollutants in different environments have centred on filamentous fungi which is opposed to these study which assessed the primitive lower fungi, aquatic phycomycetes, the study is therefore a wakeup call for further studies on the use of aquatic phycomycetes as bioremediation agent.

References:

1. Adekunle, A. A. and Adebambo, O. A. (2007). Petroleum Hydrocarbon Utilization by Fungi Isolated From *Detarium senegalense* (J. F Gmelin) Seeds. *Journal of American Science* 3: 69-76.
2. Adekunle, A. A., Uaboni-Egbenni, P.O. & Ajayi, T. (2004). Biodegradation of petroleum products by *Saccharomyces cerevisae* isolated from the Lagos, lagoon. *Journal of Bioremediation and Biodegradation*, 17, 83- 94.
3. Alabi, R.O. (1971). Seasonal periodicity of Saprolegniaceae at Ibadan, Nigeria. *Transaction of British Mycological Society* 56: 337-341.
4. Atagana, H.I., Haynes, R.J. & Wallis, F.W. (2006). Fungal bioremediation of creosote-contaminated soil: A laboratory scale bioremediation study using indigenous soil fungi. *Water Air Soil Pollution*, 172, 201-219.

5. Atlas, R.M. (1995). Petroleum biodegradation and Oil Spill bioremediation *Marin Pollution Bulletin* 31: 178-182.
6. Branislav, R. (2005). Five Serbian reservoirs contain different fungal propagules. *Mycologia,* 97(1):50-56.
7. Dibble, J.T. and Bartha, R. (1979). The effect of environmental parameters on the biodegradation of oily sludge. *Applied Environmental Microbiology* 37: 729-739.
8. El-Hissy, F.T., El-Zayat, S.A. & Massoud, M.S. (2000). Monthly vertical fluctuations of aquatic fungi at different depths in Aswan high Dam Lake, Egypt. In Aquatic mycology across the millennium (eds K.D. Hyde., W.H. HO and S.B. Pointing). *Fungal diversity,* 5:165-173.
9. Gesinde, A.F., Agbo, E.B., Agho, M.O. & Dike, E.F.C. (2008). Bioremediation of Some Nigerian and Arabian Crude Oils by Fungal Isolates. *International Journal of Pure and Applied Sciences,* 2: 37-44.
10. Hadibarata, T. & Tachibana, S. (2009) Microbial Degradation of Crude Oil by Fungi Pre-Grown on Wood Meal. In book: Proceedings of Interdisciplinary Symposium on Environmental Science-Environmental Research in Asia. (Edition 2), Terrapulo, pp317-322.
11. Husaini, A., Roslan, H.A., Hii, K.S.Y. & Ang, C.H. (2008). Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites. *World Journal of Microbiology and Biotechnology,* 24: 2789-2797.
12. Khulbe, R.D. (2001). A manual of aquatic fungi (Chytridiomycetes and Oomycetes). Daya publishing house, Delhi. 255pp.
13. Marano, A.V., Barrera, M.D., Steiow, M.M., Donadelli, J.L. & Saparrat, C.M.N, (2008). Frequency, abundance and distribution of zoosporic organisms from Las Canas stream (Buenos Aires, Argentina) *Mycologia,* 100:691-700.
14. Marano, A.V., Barrera, M.D., Steiow, M.M., Donadelli, J.L. and Saparrat, C.M.N. (2008). Frequency, abundance and distribution of zoosporic organisms from Las Canas stream (Buenos Aires, Argentina) *Mycologia* 100:691-700.
15. Nascimentol, C. A., Eduardo, P. C. G. and Pires-Zottarelli, C. L. A. (2011). Occurrence and distribution of zoosporic organisms in water bodies from Brazilian cerrado. *Mycologia* 103(2): 261-272.
16. Nilanjana,D. and Preethy, C. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnolology Research International* (941810):13pp.
17. Obire, O. & Anyanwu, E. C. (2009). Impact of various concentrations of crude oil on fungal populations of soil. *International Journal of Environmental Science and Technology* 6: 211-218.

18. Obire, O. and Putheti, R.R. (2009). Fungi in bioremediation of oil polluted environments, pp 1-10. http:// energy .sigmaxi.org/wpcontent/uploads/2009/09/obire_putheti_bioremediation.pdf..
19. Ogbonna, C.I.C. & Alabi, R.O. (1991). Studies on species of fungi associated with mycotic infections of fish in a Nigerian fresh water fish pond. *Hydrobiologia* 220:131-135
20. Okerentugba, P.O. & Ezeronye, O.U. (2003). Petroleum degrading potentials of single and microbial cultures isolated from rivers and refinery effluent in Nigeria. *African Journal of Biotechnology* 2(9):288-292.
21. Romero, M.C., Urrutia, M.I., Reinoso, H.E. & Kiernan, M.M. (2010). Benzo[a]pyrene degradation by soil filamentous fungi. *Journal of Yeast Fungal Research* 1: 025-029.
22. Saraswathy, A. & Hallberg, R. (2002). Degradation of pyrene by indigenous fungi from a former gasworks site. *FEMS Microbiology Letters* 210: 227-232.
23. Sparrow, F.K. (1960). Aquatic phycomycetes (2nd ed). University of Michigan press. Ann Arbor. 1187pp.
24. Trifa, K. F. and Adiba, S.A. (2011). An ecological study on the occurrence and distribution of aquatic fungi in Sarchnar water spring within Sulaimani province, Kurdistan region of Iraq. *Journal of Al-Nahrain University* 14(4): 137-145.
25. Vashishta, B.R. and Sinha, A.K. (2002). Botany for degree students-Fungi. S. Chandand company ltd, New Delhi, India.676pp.
26. Westlake, D.W., Jobson, A., Phillippe, R. and Cook, F.D. (1974). Biodegradability and crude oil composition. *Canadian Journal of Microbiology* 20(7): 915-928.
27. Yerima, M.B., Balogun, A.A., Farouq, A.A. & Muhammad, S. (2009). Laboratory based degradation of light crude oil by aquatic phycomycetes. *African Journal of Biotechnology* 8 (16), 3851-3853.
28. Zajic, J.E. and Supplisson, B. (1972). Emulsification and degradation of Bunker C fuel oil by microorganisms. *Biotechnology and Bioengineering* 14 (3), 331-343.