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Bioremediation of Anthracene by Aspergillus niger and Penicillium Funiculosum

Ihsan Flayyih Hasan AI-Jawhari
Department of Environment and Pollution, Marshes Research Centre, Thi-Qar University, Iraq
dr.ihsan_2012@yahoo.com

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Abstract

Five filamentous fungi isolated from the upper surface of sediments of center marshes (Abu-Sabayat marshe) in four stations S1,S2,S3,S4 in AI-Nasiriya governorate by using dilution method. The results showed that Aspergillus niger and Penicllium funiculosum were more common fungi among other fungi isolated in this study with high frequency. The appearance percent of A. niger, Penicllium funiculosum reached to 100%, and A.versicolor appear with 75%, but the percent of A.ostianus reached to 62.5%, as well as the results obtained that A. fumigatus appear with low percent (25%). The results showed that the different sites in present study were no high effect on fungal diversity in a sediments of marshe because the similarity of environmental conditions in study sites. The results showed that A.niger, P.funicculosum were the highest resistance to anthracene. The colony diameters were calculated in 2, 5, 7 days in the 2.0 mg of anthracene. Statistical methods obtained no significant differences between fungi, but obtained significant with the time of incubation with anthracene . The dry weight of both fungi were also resistant to anthracene. Mixed to fungal was appear A.niger + P.funiculosum the highest resistance to 2.0 mg anthracene in mineral salts medium. Also the mycelial dry weight of axenic culture of of P.funiculosum was higher than control ,but the mycelia dry weight of of axenic culture of A.niger was lower than control. The statistical methods obtained non significant differences between fungi in polluted liquid medium with anthracene. The results showed that both fungi can degraded anthracene to other compounds.

Keywords: Anthracene, fungi, biodegradation, solid media, liquid media, Sediment.

Introduction

PAHs are one group to toxic ecosystem and accumulated in soil and aquatic systems, food chain[1]. Chaudhary G.S.[2] that PAHs are hydrophobic compounds, whose found in environment is due to low dissolved in water, and anthracene with other PAHs is persistent and farmful soil contaminant[1]. Among the various PAHs emitted from the fuel combustion anthracene, a tricyclic aromatic hydrocarbon causes many problems associated with health and environmental impact. It is released due to incomplete combustion of fuels present in automobiles[3].

Anthracene show toxicity to phytoplankton, zooplankton, algae fish, and other aquatic organisms, also shows accumulation in the food chain[4]. PAHs show carcinogenic, mutagenic and other toxic charactersitic[5]. As well as PAHs causes dangerous effect to the ecosystem and human health[6]. Anthracene is one between polycyclic aromatic hydrocarbons and was selective in present investigation because anthracene was relative toxicity, and it is a probable inducer of tumors[7]. However PAHs may expose to chemical oxidation, photocatalytic, bioaccumulation, evaporation and adsorption, biological remediation is the biger process on the effecting on PAHs persistence in environment (in both terrestrial and aquatic ecosystem)[6,8,9]. In last decade, bioremediation, which is effected and economic, efficient, friendly alternative method to the environment than other removal processes such as chemical, mechanical and physical methods, to clean up environment[10]. Although the existing of microorganisms are a primary limiting factor to removal PAHs from ecosystem[11]. The sediments are a reservoir for PAHs accumulation, and in the same time the concentration of PAHs depend on the atmospheric deposition petroleum, transportation, sewage disposal or boating[12]. Many literature shown that filamentous fungi are playing in important role to removal PAHs, and even more effectively than some bacteria[13,14]. Among of non-ligninolytic fungi that *Penicillium simplicissmum* showed an degradation of anthracene[15].

The purpose in present investigation was to study filamentous fungi (*A.niger* and *P. funiculosum* to degrade anthracene. The selected these fungi in this study because these fungi were highly frequency among other fungi isolated in this investigation, and of their ability to remove some xenobiotic compounds. This study is the first in Iraq on fungi isolated from the surface of sediments in Abu- Sabayat marshe, south of Iraq.

Materials and Methods

Chemicals: All chemicals used in present investigation are bring from BDH Co. , and the purity of this chemicals are 99.99%.

Collection of sediments samples: Eight sediments samples were collected from four stations S1,S2,S3,S4 during December from the center marshes (Abu–Sabayat marsh), in AI-Nasiriya governorate, South of Iraq (Figure-1). The methods used for collection of the sediment samples were the same as described previously by Hohnk W.[16].

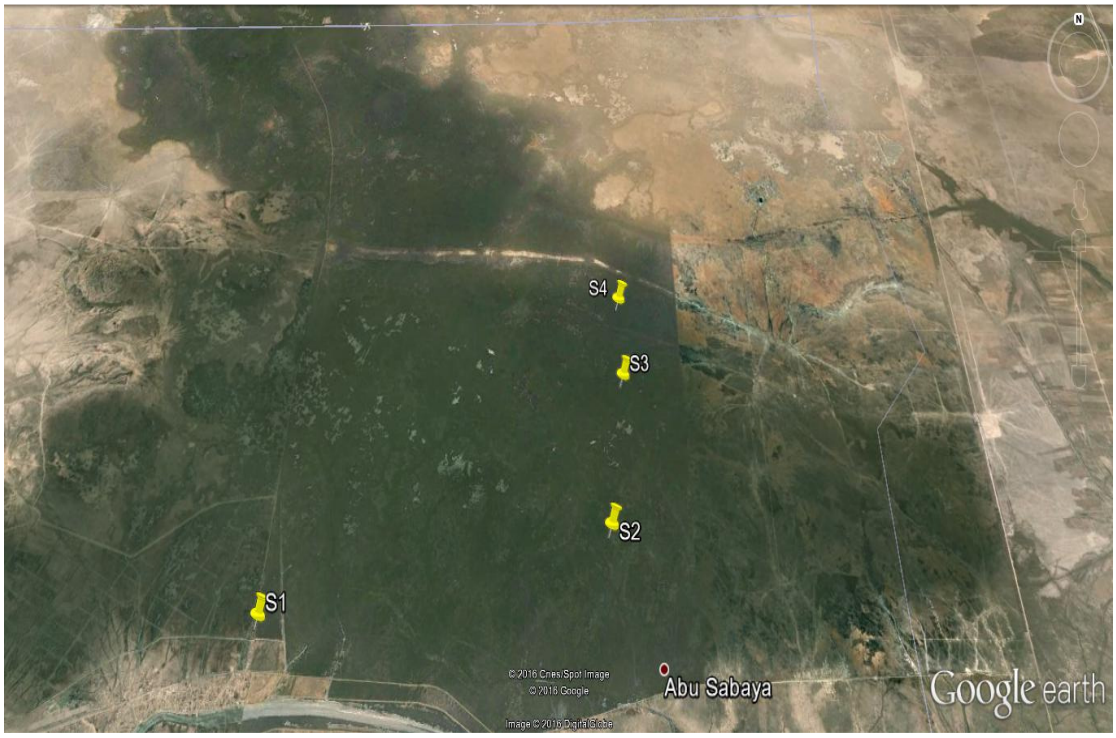

Figure-1
Digital image from Google earth showed collected samples locations (S1, S2, S3, S4) in Abu–Sabayat marshe

Isolation of fungi: One technique are applied to isolated fungi from the surface (15-30 cm) depth of sediments samples by using dilution plate method[17]. Two media were used in this study, Potato dextrose agar (PDA), and Mineral salts medium (MSM). Each media were supplemented with 250 mg^{I-1} chloramphenicol to kill bacteria. One lopeful were transfered from the plates and examined under a dissecting microscope. Mineral salts medium containing (gI^{-1}): K_2HPO_4, 1.71; KH_2PO_4, 1.32; $NaNO_3$, 0.42; $MgSO_4.7H_2O$, 0.42; $CaCl_2$, 0.02 was used for fungi growth.

General and specific taxonomic references were used for the identification for fungal species 18,19. Anthracene at 2.0 mg was used as a sole of carbon.

Effect of anthracene on isolated fungi in solid medium: The growth examined to find the resistant isolated fungi to anthracene in solid medium, and the growth were comparing with control. 2.0 mg anthracene in 0.3 mI acetone was added to warm PDA before solid in all plates. Dishes without added anthracene were used in control plates. This experiment was done duplicate. All dishes were inoculated with 5mm from 7 day old colonies. The dishes were transferred to incubator with 25co. Colony diameter of all fungi under study were calculated after 7 days and compared with control.

Effect of anthracene on isolated fungi in liquid medium: The growth examined to find the resistant isolated fungi to anthracene in liquid medium. The growth were comparing with control, as a weight of mycelium. 2.0 mg of anthracene was added as a source of carbon to 250 mI mineral salts medium. The liquid mineral salts medium were inoculated with 5mm disk from the mycelial of the old 7 days. The control flasks were not inoculated with mycelial of fungi colony isolated. All flasks were covered with non- absorbent cotton wool and incubated 7 days in $25C^{o}$. The flasks were shaken manually to mixed content. The mycelial dry weight of isolated fungi was calculated by using sensitive balance after filtration through Whattman NO.1 filter paper. Hydrogen ion concentration was calculated by using pH meter.

Biodegradation of Anthracene: Calculation of residual anthracene was done by using quantatitative analysis by Gas chromatography with some modification of method 6 after 28 days incubation. Residual anthracene were extracted with hexane (1:2), and centrifuged for 10 min at 10000g, after separation 1 mI of hexane was filtered with milipore filters paper (0.45 µm) and transported into a sterile vial for evaporation of hexane. After complete evaporation of hexane, 1 mI of acetonittrile was added to the residue and the remaining anthracene was analyzed by Gas chromatography (GC-FID

Shimadzu 2014), the injector volume was 1µI and the oven temperature started at 60C^{o} (1min), and increased by 25C^{o} min^{-1} to 150C^{o}, and 10min^{-1} to 260C^{o} field for to 20 min), and increased to 270C^{o} (held for 20min), the carrier gas was 1 mI min^{-1} helium, in the same time the remaining anthracene was determined by Infrared spectroscopy (FTIR).

Statistical Analysis: All applications were analysis by using (ANOVA) in program SPSS (version 10.0).

Results and Discussion

Isolation fungi: Five filamentous fungi isolated from the upper surface of sediments of center marshes (Abu-Sabayat marshe) in AI-Nasiriya governorate in four satations Table-1. Table-2 showed the total numbers of fungal genus in all samples with frequency to all genus in a sediments, Table-3 showed that *Aspergillus niger* and *Penicllium funiculosum* were more common fungi among other fungi isolated in this study, with high frequency. Table-3 obtained that the appearance percent of *A. niger* and *Penicllium funiculosum* reached to 100%, also the appearance percent of *A.versicolor* reached to 75%, and with *A.ostianus* reached to 62.5%, but the appearance percent of *A.fumigatus* reached to 25%. The presence of these fungi due to that *A.niger* was known as environmental common species 20. These results were different to the findings of Al-Jawhari I.F.[21] which showed that the appearance percent of *A.fumigatus*, *P. funiculosum* reached to 83% in Suq-AI Shuyukh marshes and also different with study of Mohammed A.R. et.al.[22] which showed that four fungi isolated ,these fungi were *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium chlamydosporum* and *Aspergillus terreus* from soil polluted with anthracene. The differences of stations in present study are no high affected in fungi diversity in sediments because the similarty of environmental conditions such as temperature, salinity, hydrogen ion concentration and found of *Phragmites sp.* and *Typha sp.* plants. However after the war 2003 in Iraq, the marshes were restoration of waters and human activities were increases with specific used to catch boats then increases pollutants in these sites with hydrocarbons pollutants and probability to reach these compounds to waters from plants after dead and degradation. And in the same time the southern marshes of Iraq were a natural filter of agriculture, industrial pollutants residue when reached these pollutants with Tigris and Eupharties waters. However fungi play an important role to degradation during by excretion extracellular enzymes such as cellulose, lignin and other compound. Also crude oil, insecticides and herbicides were degradation by *A.niger*, *F.solani*, *Penicillium sp.* and *Trichoderma lignorum*[22-25].

Table-1
List of fungi isolated from the upper surface of sediments in different stations in Abu- Sabayat marshe

Fungi Species	Stations			
	S1	S2	S3	S4
A.niger Tighem	+	+	+	+
A.versicolor (Vuill)Triqboschi	+		+	+
A.fumigatus Fresenius	-	-	+	-
A.ostainus	-	+	-	+
P.funiculosum Thom	+	+	+	+

(+): Funguses appear, (-): Fungus not appears.

Table-2
Total numbers of fungal genus in all samples with frequency to all genus in a Sediments

Genus of fungi	Total numbers of genus in all samples	Frequency %
Aspergillus	10.0 x 10^{4}	37.0
Penicillium	17 x 10^{4}	62.9

Total numbers of all fungi genus 2.7 x 10^{5}.
Genus frequency = Total calculated of genus in all samples / Total calculated to all genus in all samples x 100.

Fungal growth ability under anthracene pollution in solid and liquid media: The capacity of fungi under study to grow with 2.0 mg of anthracene and was done by calculated the colony diameter (Figure-2). The results showed that both fungi *A.niger, P.funicolsum* are resistant to anthracene pollution. Also the results showed that *P.funicculosum* was larger resistance to anthracene pollution, and the diameter of colony is equal with control (8.5 cm) after 7 days incubation. The colony diameters was examined in 2, 5 7 days in the 2.0 mg of anthracene. Statistical methods obtained no significant differences between fungi, but obtained significant with the time of incubation.

These observations due to that these fungi were grow well in the presence of anthracene in the solid media[8]. And in the same time the results in present study were similar with the findings of Giraud F. et al.[26] showed that anthracene was not highly effected for the selected fungi. The result in present study was different to the findings of Al-Jawhari I.F.[21] showed that the colony diameter of *P.funiculosum* reached to 3.6 cm and the colony diameter of *A.niger* reached to 8.5 cm, and the results obtained in present study also different with Wang O. et.al.[27] show that the diversity of the microorganisms population was highly decreased with the bioremediation of anthracene. The results in present study refer the adaptation of both fungi to anthracene polluted medium. It seem that anthracene, as well as other petroleum compounds can degraded by different filamentous fungi[10,28]. Petroleum hydrocarbons could not reduce the growth of fungal species in soil with petroleum hydrocarbons, and in the same time that the fungal species used oil components to support growth and crude oil pollution cause to accelerated fungal growth 29. Also the organic compounds in soil were accelerated the growth of fungi and increases excreted extracellular enzymes were produced to removal of pollution with petroleum hydrocarbons and decrease of soil pH[28]. However the results showed that the axenic culture of *P.funiculosum* is resistant to anthracene and the meycelial dry weight of this fungus reached to 5.664 when compared with control (4.482), but the mycelial dry weight of *A.niger* was lower than control. The mycelial of this fungus reached to 3.963. Also the mycelial dry weight of mixed *P.funiculosum* and *A.niger* was higher than control, and the mycelial dry weight reached to 5.740 (Table-4).

The statistical methods obtained non significant differences between fungi. No significant differences were obtained in pH value with consuming anthracene by both fungi isolated through application period (Table-5). The results showed that the pH in liquid medium inoculated with *A.niger* lower than *P.funiculosum* and control, the pH with *A.niger* reached to 5.11. The reduction in pH may be due to chemical changes of hydrocarbon compounds during precipitated by microbial enzymes 30. This result was similar to the findings of Al-Jawhari I.F.[21] also showed that the pH to *P.funiculosum* reached to 5.1 and the pH of *Fusarium solani* reached to 5.8. Many fungi can metabolise a wide range of pH. Microbial degradation produces organic acids and different compounds31. However organic acids may be reduction in pH levels[32].

Table-3
Frequency of species isolated from the upper surface of sediments of Abu-Sabayat Marshe

Fungi Species	Numbers of fungal species appear	Frequency%
A.niger Tighem	8	100
A.versicolor (Vuill)Triqboschi	6	75
A.fumigatus Fresenius	2	25
A.ostainus Wehmer	5	62.5
P.funiculosum Thom	8	100

Table-4
Ability growth of *A.niger, P.funiculosum* in treatment liquid medium with anthracene

A.niger	P.funiculosum	A.niger + P.funiculosum	Control
3.963*	5.664	5.740	4.482

(*)Non significant.

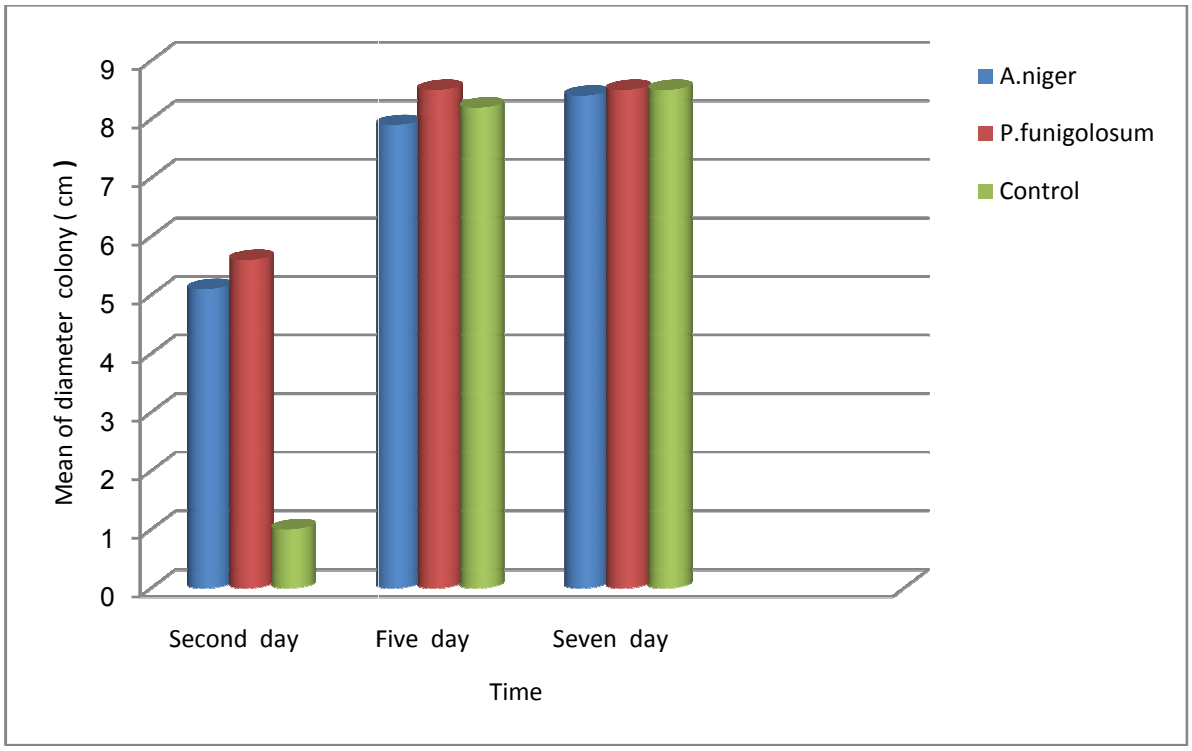

Figure-2
A bility growth of *A.niger, P.funiculosum* in treatment solid medium with anthracene

Table-5
Variation in pH in treatment liquid medium with anthracene

Time (day)	*A. niger*	*P. funiculosum*	*A.niger* + *P.funiculosum*	Control
7	6.17*	7.67	7.58	7.90
14	6.16	7.16	7.13	7.90
21	5.16	7.10	7.08	7.90
28	5.11	7.05	7.04	7.90

Biodegradation of Anthracene: Figure-4,5 showed that GC chromatograms refer the existing different metabolites when compared with anthracene standard (Figure-3), all figures showing anthracene degradation by *A.niger*, *P.funiculosum* and mixed *A.niger* + *P.funiculosum*. Different metabolites were exists but difficult identified in this study because these metabolites were not found in Iraq now. These observations referred that a degradation and transformation process was happened. Figure-4 showing that antharacene degradation when compared with standard anthracene, and the concentration of anthracene reached to 0.00047 mg and the removal percentage reached to 99.97%, this result exhibited the highest anthracene removal rates after 7 day incubation with mixed pure culture of *A.niger* and *P.funiculosum*, the peak for anthracene corresponded with the peak that eluted after 5.725 min, but the peak of three metabolites appear in retention time 5.906, 9.571, 9.646 (Figure-4). These results were similar with findings of 33 which showed that also anthracene was degraded after 15 day of incubation with *Plerurotus ostreatus* under solid-state fermentation.

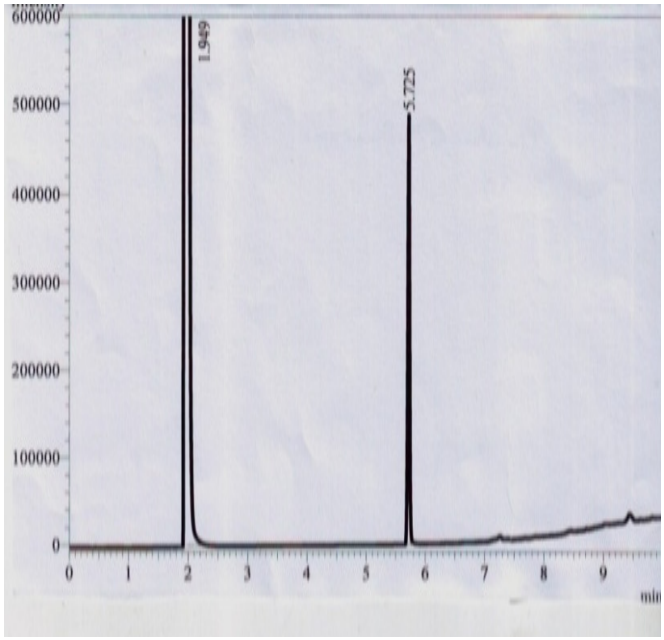

Figure-3
GC-chromatogram showed standard anthracene (control)

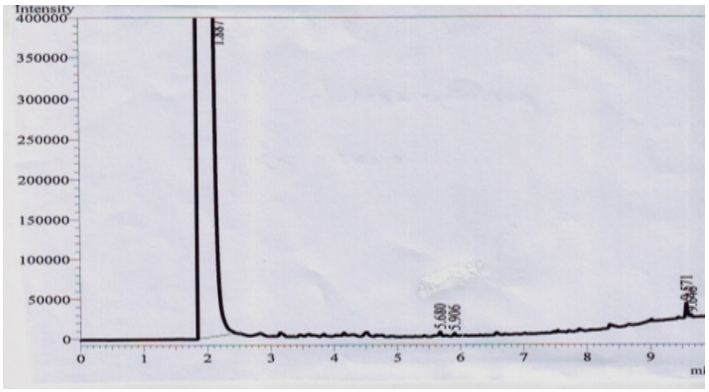

Figure-4
GC-chromatogram of amixed culture of *A.niger* and *P.funiculosum* with anthracene treatment after 7 days incubation

Figure-5 showing the highly removal of anthracene, and the anthracene was disappeared completely after 21 days incubation in mixed pure culture of *A.niger* and *P.funiculosum*, when compared with standard anthracene. GC chromatogram showed new five peaks appears, these peaks suggest formation of degradation products that until now not been identified because the metabolites of anthracene was not found in Iraq now, based on a review of bibliographic reference 34, one of the peaks may be anthracene trans-1,2-dihydrodiolalso, also Patricia et al.[15] that anthracene transformed to an throne, 9,10-anthraquinone, and finally produced phthalic acid Figure-6. Boyle D. et al.[35] reported a GC retention time and mass spectrum as an authentic standard of 9,10 anthraquinone like product resulting from the lignin peroxidase reactions.

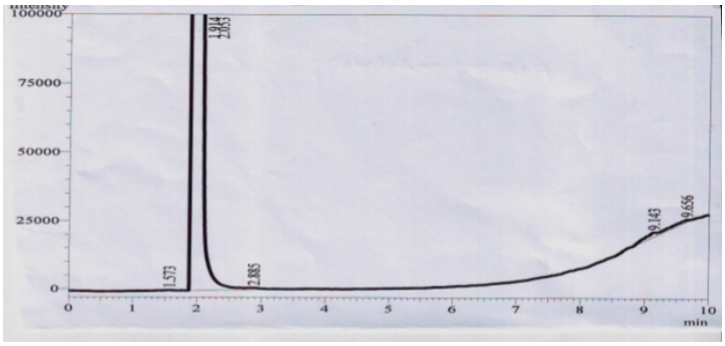

Figure-5
GC-chromatogram of amixed culture of A.niger and P.funiculosum with anthracene treatment after 21 days incubation

Figure-7 also showing biodegradation of anthracene after 21 days incubation with mixed pure culture of A.niger and P.funiculosum, and many peaks were appear in 2000 – 3500 when compared with standard anthracene (Figure-8).

These results were similar to the findings of 15 which showed that the degradation values of anthracene were ranged from 20% to the complete removal. These variation depended on different factors such as, concentration of anthracene, type of medium, pH and temperature, It was also observed that the percentage of PAH biodegradation was significantly higher in liquid media than in solid media conditions, because may be due to the decreased PAH availability in solid medium[36,37] .

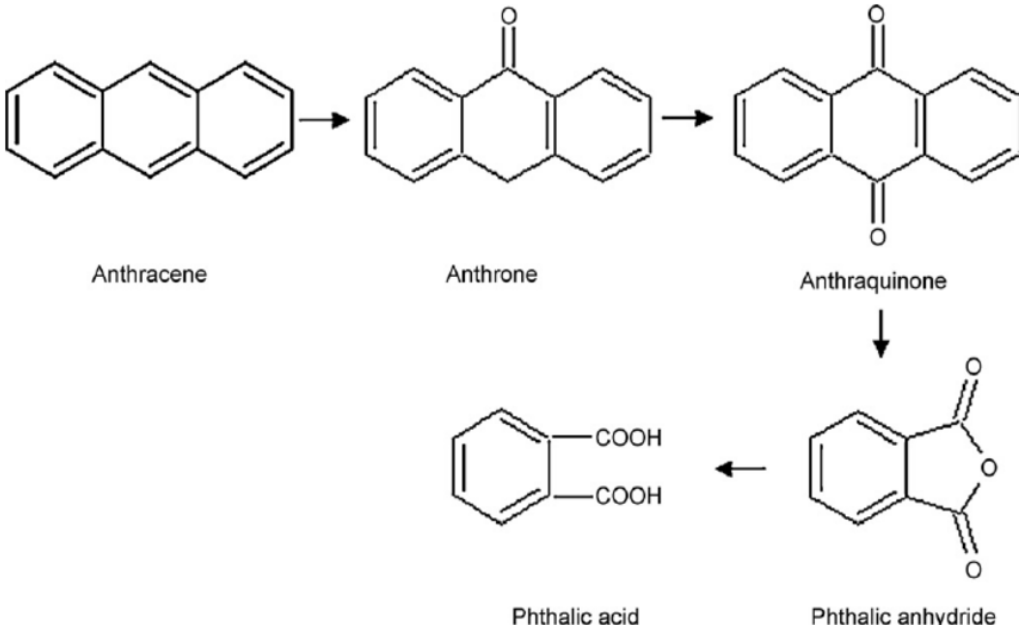

Figure-6
Proposed pathway of anthracene degradation after 21 day incubation with a mixed culture of A.niger and P.funiculosum after 21 day

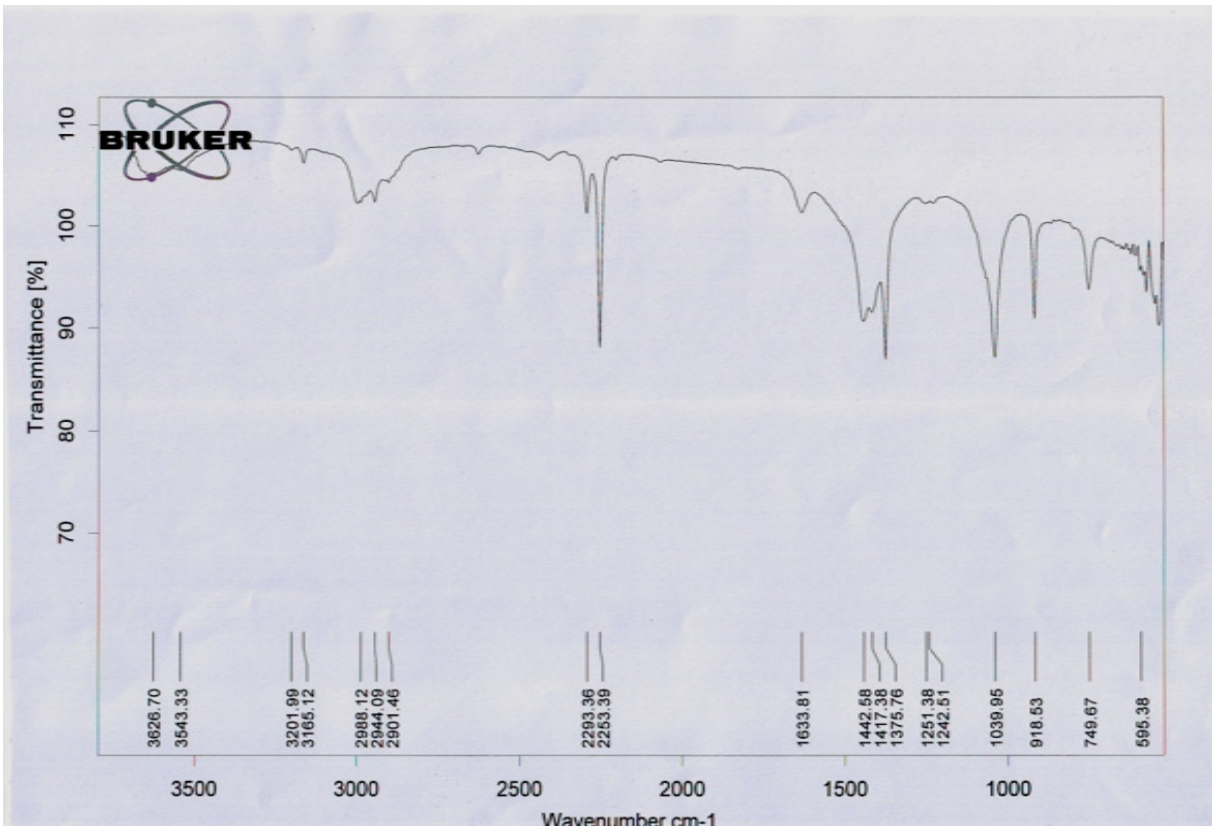

Figure-7
Bioremediation of anthracene by mixed pure culture of A.niger, P.funiculosum after 21 day incubation

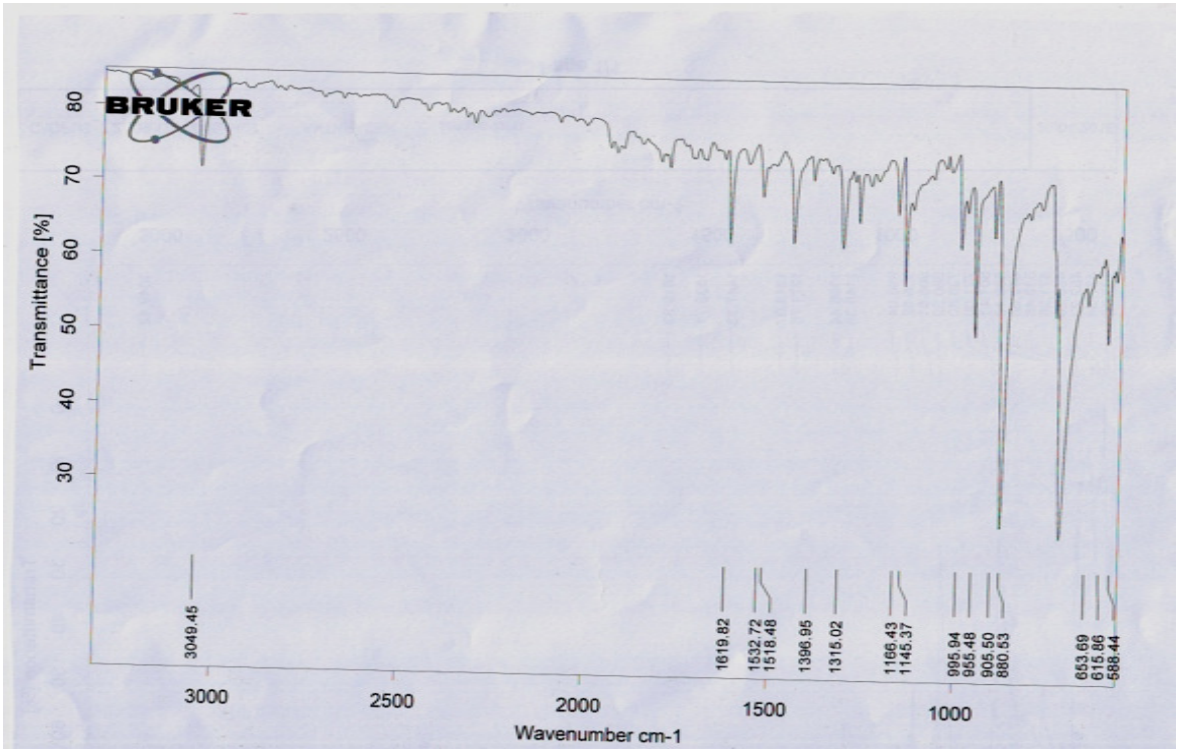

Figure-8
Anthracene (Standard)–uncirculated

Figure-9 showing biodegradation of anthracene and one peak appear in GC chromatogram after 7 day incubation with *P.funiculosum*, and the concentration of anthracene decreased to 0.000047 mg when compared with 2.0 mg control, and the removal percentage reached to 99.99%. This result exhibited the highest anthracene removal rates. Also this result was similar to the findings of Patricia J.[15] showed that *P.simlicissimum* was highly degradation of anthracene with 86%, but the results showed that *P.ostreatus* was the lowest percentage of anthracene removal with 15%, also the results in present study were the similar to the findings of Mohammed A.R. et. al.[22] showed that *Fusarium oxysporum* was ablity to degrade 78% from the initial concentration of anthracene after 7 days of inoculation, while bacteria *Pseudomonas aureofaciens* degraded 70% under the same conditions.

Figure-10 showed 4 metabolites with 4 peaks after 21 days incubation with pure culture of *P.funiculosum*, and also Figure-11 show different compounds were obtained in FTIR spectrum when compared with standard anthracene. This results explained that the biodegradation by fungi due to that fungal mycelium which has a high surface area than can maximize both mechanical and enzymatic contact with insoluble substrate such as anthracene and invade a larger volume of soil[10]. In the same time Mohammed A.R. et.al.[22] that the degradation of anthracene by fungi are more feasible than bacterial biodegradation.

Figure-12 showing the biodegradation of anthracene after 21 days incubation with pure culture *A.niger*. Two new chromatographic peaks were also observed. These new peaks suggest formation of degradation products that until now have not been identified because the metabolites of anthracene were difficultly found in Iraq now. And also Figure-13 show different compounds were obtained in FTIR spectrum when compared with standarad anthracene . Also This result show that *A.niger* was lower efficiency when compared with axenic culture of *P.funiculosum* or with mixed culture of *P.funiculosum* and *A.niger*. This result was similar to the findings of 15 which showed that the final percentage of anthracene removal obtained with other white – rot fungi *P.chrysosporium* and *I.lacteus* were higher (~ 40%) than those obtained with the non- ligninolytic fungi *A.niger* (31%) or *M.racemosus* (24%).

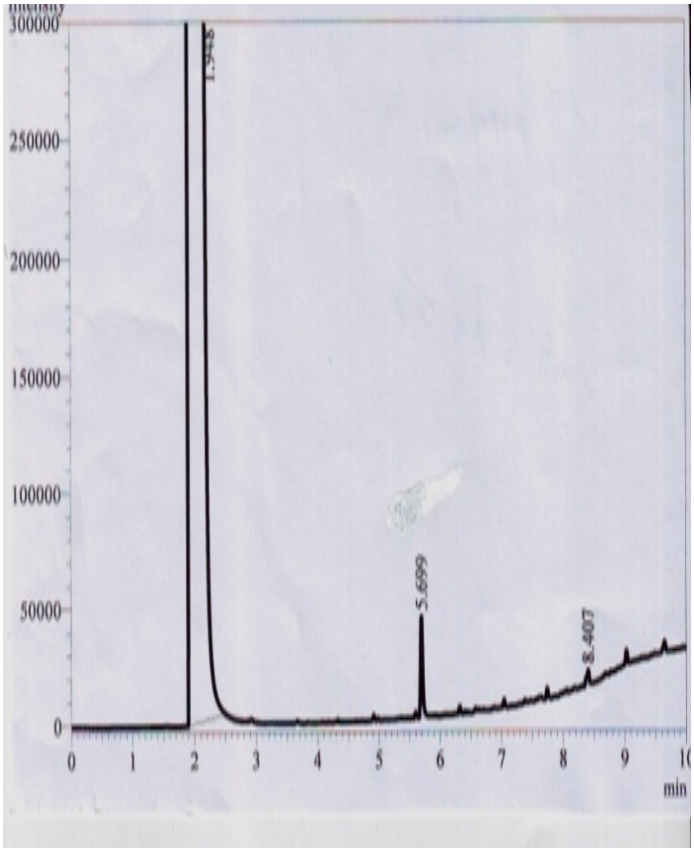

Figure-9
GC-chromatogram of axenic culture of P.funiculosum treatment with anthracene after 7 day incubation

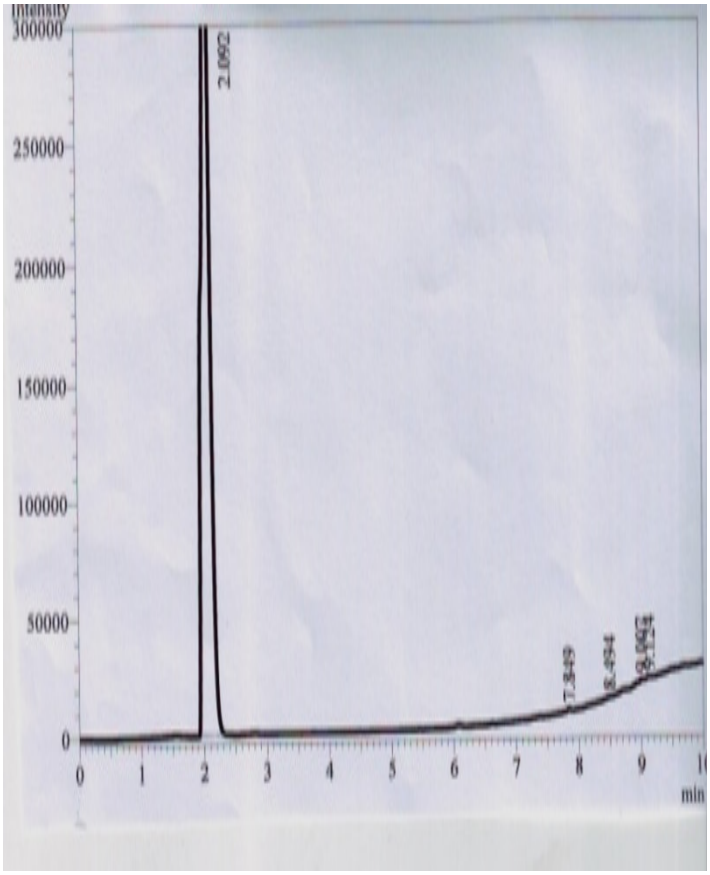

Figure-10
GC-chromatogram of axenic culture of P.funiculosum with treatment with anthracene after 21 day incubation

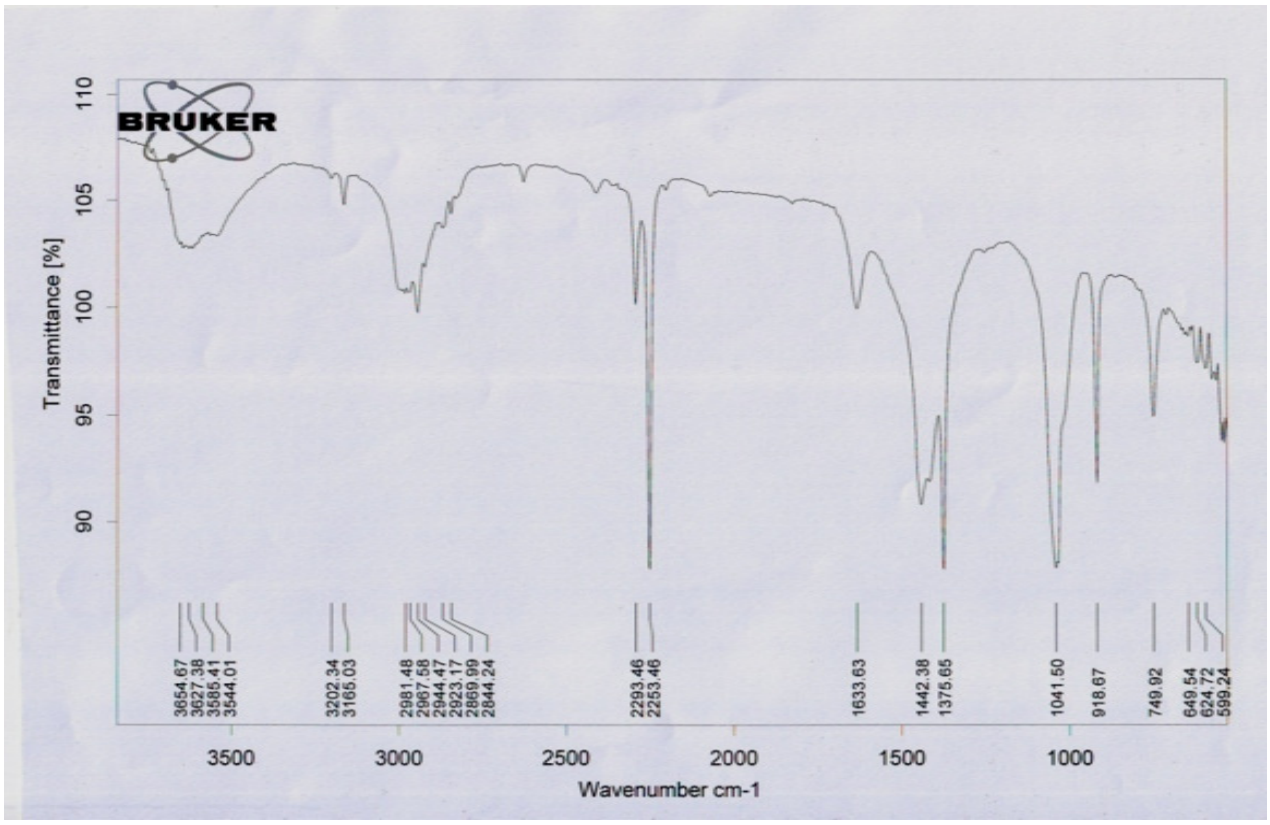

Figure-11
Bioremediation of anthracene by *P.funiculosum* after 21 days incubation

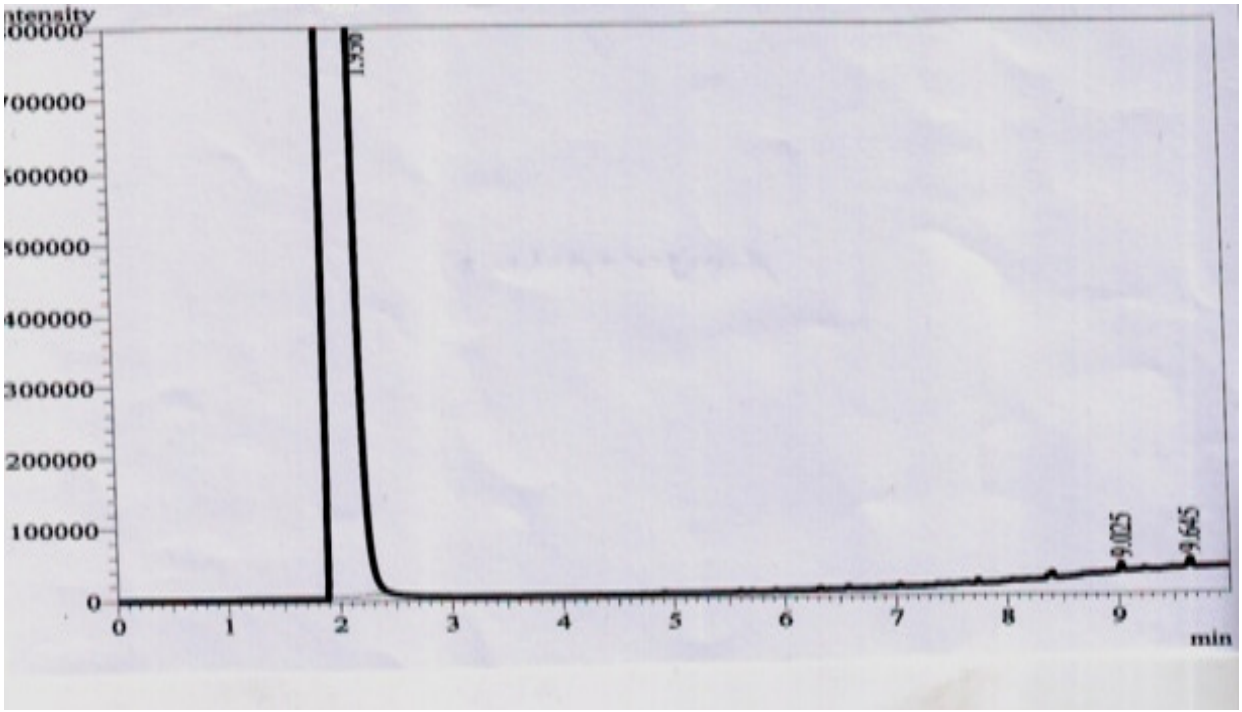

Figure-12
GC-chromatogram of anthracene after a 21 day exposure to a pure culture of *A.niger*.

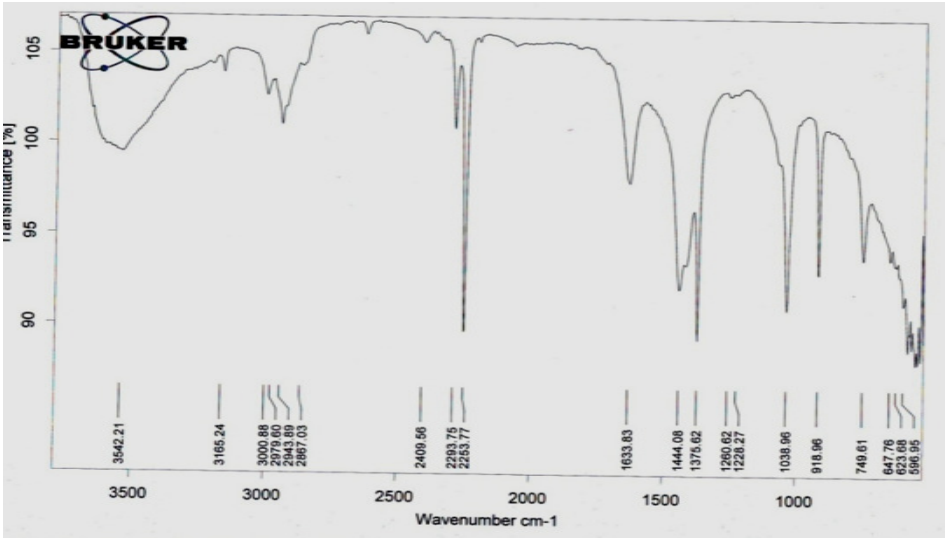

Figure-13
Biodegradation of anthracene by *A.niger* after 21 day incubation

Conclusion

The differences of stations in present study are no high effected in fungi diversity in sediments because the similarity of environmental conditions such as temperature , salinity , hydrogen ion concentration and found *Phragmites sp.*, *Typha* sp. Also the results showed that mixed pure culture from *A.niger* , *P.funiculosum* was more efficiency to removal anthracene and also the axenic culture of *P.funiculosum* was appear ability to removal anthracene higher than the axenic culture of *A.niger*. These data in the present study was advanced our knowledge of polyaromatic hydrocarbons and behavior of fungi in polluted marshes in different location , and how these fungi to breakdown or biodegradation of pollutants in environment, as well as can used these organisms to removal pollution now and also in future .

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References

1. Juhaz A.L. and Naidu R. (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo (a) pyrene. *Int. Biodeter. Biodeg.,* 45, 57-88.

2. Chaudhry G.R. (1994). Biological degradation and bioremediation of toxic chemicals. Dioscoides press, Portland, OR, USA.

3. Kanaly R.A. and Harayama S. (2000). Biodegradation of higher molecular weight polycylic aromatic hydrocarbons by bacteria. *BacterioI*., 128(8), 2059–2067.

4. Sutherland J.B. (1992). Detoxification of polycyclic aromatic hydrocarbons by fungi. *J. Indust. MicrobioI.*, 9, 53–62.

5. Crisafully R., Milhome M.A., Cacalcante R.M., Silveira E.R., Keukeleire D.D. R.E. Nascimento R.E. (2008). Removal of some polycyclic aromatic hydrocarbons from petrochemical wastewater using low–cost adsorbents of natural origin. *Biores. TechnoI.,* 99, 4515–4519.

6. Yuan S.Y., Wei S.H. and Chang B.V. (2000). Biodegradation of polycyclic aromatic Hydrocarbons by a mixed culture. *Chemo*, 41, 1463–1468.

7. Das P., Mukherjee S. and Sen R. (2008). Improved bioavailability and biodegradation of model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine origin. *Chemo.*, 72, 1229–1234.

8. Cerniglia C.E. (1993). Biodegradation of polycyclic aromatic hydrocarbons. *Curr. Opin . BiotechnoI.,* 4, 331–338.

9. Mahmood S.K. and Rama Rao P. (1993). Microbial abundance and degradation of polycyclic aromatic hydrocarbons in soil. *Bull.Environ. Contam.Toxi.,* 50(4), 486–491.

10. Potin O., Rafin C. and Veignie E. (2004). Bioremediation of an aged polycyclic aromatic hydrocarbons (PAH_s) contaminated soil by Filamentous fungi isolated from the soil. *Inter. Biodet. Biodeg.*, 54, 45–52.

11. Alexander R.R. and M. Alexander (2000). Bioavailability of genotoxic compounds in soil. *Environ . Sci . TechnoI.,* 34, 1589 – 1593.

12. Baumard P., Budzinski H., Michon Q., Garrigues P., Burgeot T. and Bellocq (1998). Origin and bioavailability of PAHs in the Mediterranean Sea from Mussel and Sediment Records. *Estua.Coast.SheI.Sci.,* 47(1),77-90.

13. Cerniglia C.E. (1997). Fungal metabolisms of PAHs. *J. Ind . MicrobioI.Bio.TechnoI.,* 19, 324–333.

14. Peng R.H., Xiong A.S., Xue Y., Fu X.Y., Gao F., Zhao W., Tian Y.S., and Yao Q.H. (2008). Microbial biodegradation of polycyclic aromatic hydrocarbons-Review. *FEMS . MicrobioI.Rev.,* 32, 927-955.

15. Patricia J ., Maria A., Olivella S., Josep C., Carles C., Laura P., Francesc C., X. and de las H. (2016). Fungal biodegradation of anthracene– polluted Cork: A comparative study. *J. Environm. Sc. HeaI*, Part A. 51(1), 70-77.

16. Hohnk W. (1972). Fungi in research methods. Inic.Schlieper (ed.)Marine biology. Univ. of Washington Seattle. Press.

17. AI-Nasrawi H. (2012). Biodegradation of crude oil by fungi isolated from Gulf of Mixico. *J. Biorem. Biodeg.,* 3(4), 1-6.

18. Pitt J. (1991). A laboratory guid to common *Penicillium* species. *common. Sci. Indus Res*. Org Division of Food processing.

19. Klich M. and Pitt J. (1992). A laboratory guid to the common *Aspergillus* species and their teleomorphs. *common. Sci. Indus Res Org.* Australia.

20. Brix H. (1997). Do mycrophytes play a role in constructed treatment wetlands. *Water. Sci. TechnoI.,* 35, 11-17.

21. AI-Jawhari I.F. (2015). A bility of some fungi isolated from a sediment of Suq 5. - AIShuyukh marshes on biodegradation of crude oil. *International J. Curr. MicrobioI. AppI. Sci .,* 4(1), 19-32.

22. Mohammed A.R., Hashem A.M., Amin M.A. and N.H. Rfky. N.H (2012). Immobilization and surfactant enhanced anthracene biodegradation in soil. *J. Amer. Sci.,* 8 (3) , 596–602.

23. AI- Jawhari I.F. (1998). A Study of fate herbicide propanil in rice field at AI-Qadisiya governorate and its effect on some water and soil microorganisms. Ph.D. Thesis, AI-Mustansiriya University, Iraq.

24. Ravelet C., Krivobok S., Sage L., and R. Steiman R. (2000). Biodegradation of Pyrene by sediment fungi. *Chemo.*, 40(5), 557–563.

25. Mtui G.Y. (2012). Lignocellulolytic enzymes from tropical fungi: Types, substrate and applications. *Sci. Res. Ess.* 7(15), 1544–1555

26. Giraud F., Guiraud P. Kadri M., Blake G. and R. Steiman (2001). Biodegradation of anthracene and fluoranthene by fungi isolated from an experimental constructed wetland for wastewater treatment. *Wat. Res.,* 35, 4126–4136.

27. Wang O., Zhang S.Y., Zou L., and Xie S.G. (2011). Impact of anthracene addition on microbial community structure in soil microcosms from contaminated and uncontaminated sites. *Biom. Environ. Sci.,* 24 (5), 543-549.

28. Atagana H.I. (2006). Biodegradation of polycyclic aromatic hydrocarbons in contaminated ation and bioaugmentation. *MicrobioI. Soil by BiostimuI. BiotechnoI.,* 22, 1145–1153.

29. Mohsenzadeh F.C., Rad A. and Akbari M. (2012). Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum–polluted soils. *Iranian J. Environ.l . HeaI. Sci. Eng* .

30. Atlas R.M. and Bartha R. (1972). Microbial degradation of oil pollutant workshop La. State Union PubI. NO. LSUSG-73- 01. 283-289.

31. Nwachukwu S.U. and Ugoji. E.O. (1995). Impact of crude petroleum spills on microbial communities of tropical soils. *Int. J. Environl.Sci.,* 21(1). 169-175.

32. Oboh O.B., Ilori M.O., Akinyemi J.O. and Adebusoye S.A. (2006). Hydrocarbon degrading potential of bacteria isolated from a Nigerian Bitumen (Trasand) Deposit. *Nature and Science,* 4(3), 1-57**.**

33. Vargas M.C., Rodriguez R., Sanchez F., and Ramirez N. (2001). Biological trans-formation of anthracene in soil by *Pleurotus ostreatus* under solid-state fermentation conditions using wheat brain and compost. *Ct. F. Cien. TechnoI.Futu.,* 2(2), 43- 50.

34. Bezalel L., Y. Hadar Y. and Cerniglia C.E. (1996). Mineralization of polycyclic aromatic Hydrocarbons by the white rot fungus *Plerurotus ostreatus*. *APPI. Environ. MicrobioI.,* 62(1), 292–295.

35. Vazquez–Duhalt R. and Westlake D.W. (1994). Fedorak M., Lignin peroxidase oxidation of aromatic compound in system containing Org. Solvent. *APPI. Environ. MicrobioI.,* 60(2), 459–466.

36. Boyle D., Wiesner C. and Richardson,A. (1998). Factors affecting the degradation of Polycyclic aromatic hydrocarbons in soil by white rot fungi. *Soil. BioI. Biochem.,* 30, 873–882.

37. Yuan S.Y., Shiung L.C. and Chang B.V., (2002). Biodegradation of polycyclic aromatic hydrocarbons by inoculated microorganisms in soil. *Bull. Environ. Contam. ToxicoI,* 69, 66–73.