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Biodegradation of Some Petroleum Hydrocarbons by Fungi Isolated from Gulf of Suez

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ABSTRACT

Petroleum hydrocarbons are one of the main pollutants which affected directly on the Gulf of Suez environment due to petroleum products deliveries and production as well as fuel combustion emissions from shipping activities and refineries. There are several Methods for treatment of hydrocarbons such as physical, mechanical, chemical and biological methods. Broth media containing ACF (accommodated fraction), WSF (water soluble fractions) or anthracene were used separately as a sole carbon source. 17 fungal species were isolated from water and sediment of three selected stations (Port-Tawfik, El-Ziaytia and Attaka), then screened in each substrate. Capillary gas chromatography (CGC) analysis used to chemically profiling each substrate after inoculation. Our results showed that, *Aspergillus flavus* was the most effective of degradation ACF reach to (98.79 %). In case of WSF the isolate *Penicillium chrysogenium* was the highest percentage of degradation (98.53 %). Anthracene degradation after 2 weeks recorded (56.08 %) by using *Cladosporium* sp. In conclusion, the Gulf of Suez contain several promising fungal species that could be used in biodegradation of petroleum hydrocarbons as a save alternatives in marine ecosystem.

Keywords: Fungi biodegradation, Petroleum hydrocarbons, Anthracene, Capillary gas, chromatography, Gulf of Suez.

INTRODUCTION

The term petroleum has been used as a synonym to crude oil which contains various hydrocarbons compounds. The most hazardous fractions of petroleum hydrocarbons, to the environment, is the polynuclear aromatic hydrocarbons (PAHs). These compounds are organic chemicals composed of fused benzene rings found naturally in crude oil and also formed during incomplete combustion of coal, oil, petrol and wood (Fetzer, 2000).

They are persistent, and may result in sublethal effects of marine biota such as inhibited growth, abnormal cellular development, and prevalence of chronic diseases, reproductive impairment, and reduced life span (Neff, 1979).

Environmental pollution by petroleum hydrocarbons is the most common site pollution issue encountered by environmental professionals due to the increase in demand for crude oil as a source of energy and as a primary raw material for industries, resulted in an increase in its production, transportations and refining. Followed by a problem was created by non-safe residuals in the environment (Plice, 1948; Gutnick and Rosenberg, 1977; Obire and Wemedo, 1996; and Obire and Amusan, 2003).

Biodegradation process is eco-friendly remediation technique to remove oil pollution. Fungi have several advantages than other microorganisms in biodegradation because of their ability to cultivate on a large group of substrates. They also produce extracellular enzymes, which can penetrate contaminated soil and remove pollutants (Messias et al., 2009). Fungal enzymes have the ability to degrade PAHs are cytochrome P450 monooxygenases, dioxygenases, proteases and lipases (Da Silva, Balaji *et al.*, 2014). Obire *et al.*, 2008 reported that species of *Aspergillus*, *Penicillium*, and *Cunninghamella*, are more efficient to transformation of a PAHs involved oxidation by a cytochrome P450 monooxygenase.

Sutherland *et al.*, (1995); Cerniglia and Sutherland, (2010) also found that fungi are responsible for the first attack of the anthracene aromatic rings and metabolized PAHs with 2 to 5 aromatic rings, and the interaction between microorganisms and hydrocarbon is variable from region to region, depending upon the quality and quantity of both.

Several locations are polluted in the Gulf of Suez due to different activities of petroleum hydrocarbons, as oil refineries, shipment activity and many industries at the western side of the gulf. The application of indigenous microorganisms for the bioremediation of oil-polluted in this area is required. Therefore, the aim of this study was to isolate local fungal species and test their efficiency in laboratory for the biodegradation of petroleum-pollution in seawater and sediments. Using these compounds water soluble fractions of crude oil (WSF) and accommodated fraction of crude oil (AFC) and anthracene as a sole source of carbon.

MATERIALS AND METHODS

Description of study area

The investigated area (Suez Bay) is located between longitudes 32° 32' 07" E. and latitudes 29° 53' 55" N. The bay is shallow extension of the Gulf of Suez, roughly elliptic in shape, with its major axis in the NE - SW direction (Fig. 1). The average length along major axis is about 13.2 km; its average width along minor axis is about 8.8 km. The mean depth is 10 m, and the horizontal surface area is about 77.13 km2. The bay is connected to the Gulf of Suez through most of its south eastern side, where a channel is dredged to depth of 20 m to serve navigation purposes, and it is connected to

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the Suez Canal by a dredged channel of 12 m depth through the north eastern side of the bay (Meshal, 1967).

The northern part of the bay is occupied by the city of Suez. The Suez Bay has two sources of water, The Suez Canal and Gulf of Suez waters. Three stations were selected in the marine environment of the Bay in the Gulf of Suez which is subjected to mixed sources of oil pollution. In particularly, at the north-western side of the Bay Port-Tawfik (1), El-Zaiytia (2) and Attaka (3), respectively.

Sampling

Water and sediments samples of the Gulf during winter season 2016 were collected by screw-capped sterile glass bottles, and stored at 40^{o}C till isolation of fungi in the Lab. In addition to measure the physico-chemical parameters (water temperature, salinity, pH, DO, and BOD) and nutrient salts (nitrite, nitrate, ammonia and phosphate) according to Parsons *et al.*, (1984) and APHA, (2005).

Sources and preparation of test compounds

The test compound Anthracene was obtained from Sigma-Aldrich CAS 120-12-7, molar mass178.23 g/mol. Crude oil was obtained from oil company (Suez Company of Petroleum Refinery) in stoppered to prepare ACF and WSF. A sample of crude oil (500 ml.) was slowly mixed with seawater (500 ml.); the crude oil-water mixture was stirred slowly for 24 hours with a magnetic stirrer. This was to enhance the dissolution in the water of the water-soluble components of the crude. The mixture was made to stand for 3 hours before it was poured into the separating funnel and allowed to stand overnight so as to obtain a clear oil-water interphase. The upper and lower layer of water, containing the ACF and WSF (accommodated fraction and water soluble fraction) of crude were decanted into a clean round bottom flask with stopper, and used as 100% strength for each sample (Anderson *et al.* 1974).

Selective isolation of fungi from water and sediment

Water and sediment samples were collected from Port-Tawfik, El-Zaiytia and Attaka in stoppered can then transported to laboratory. Fungal isolation was carried out according to Chikere and Azubuike (2014), using Czapek's broth (Smith and Dawson, 1944) contain (crude oil) which obtained from oil company (Suez Company of Petroleum Refinery) or anthracene (Sigma aldrich) 0.025 g (salt or soluble in ethanol). Or WSF water soluble and accommodated fractions which prepared according to (Anderson *et al.*, 1974). Briefly, One ml of either water or 1 g of sediment samples was aseptically removed with a sterile pipette and spread on petri dishes 5 replicate for each treatment. All plates were incubated at 27^{o}C for 7 days.

Selected fungal colonies were purified in order to taxonomic identification using a phenotypic approach down to the species level on microscopical characteristics of fungal isolates by standard media based on the following identification keys: Pitt (1979), for *Aspergillus*; Ellis (1971) for *Dematiaceous hyphomycetes*; Booth (1971) for *Trichoderma*.

Screening of fungal biodegradative abilities

Twenty three (17 only in table 1) fungal isolates, belonging to 11 (12 species in table 1) species were tested. Five of them were tested with WSF and ACF. Flasks were incubated at (27^{o}C) for (5 weeks). Then, the sample was extracted at fixed intervals after first

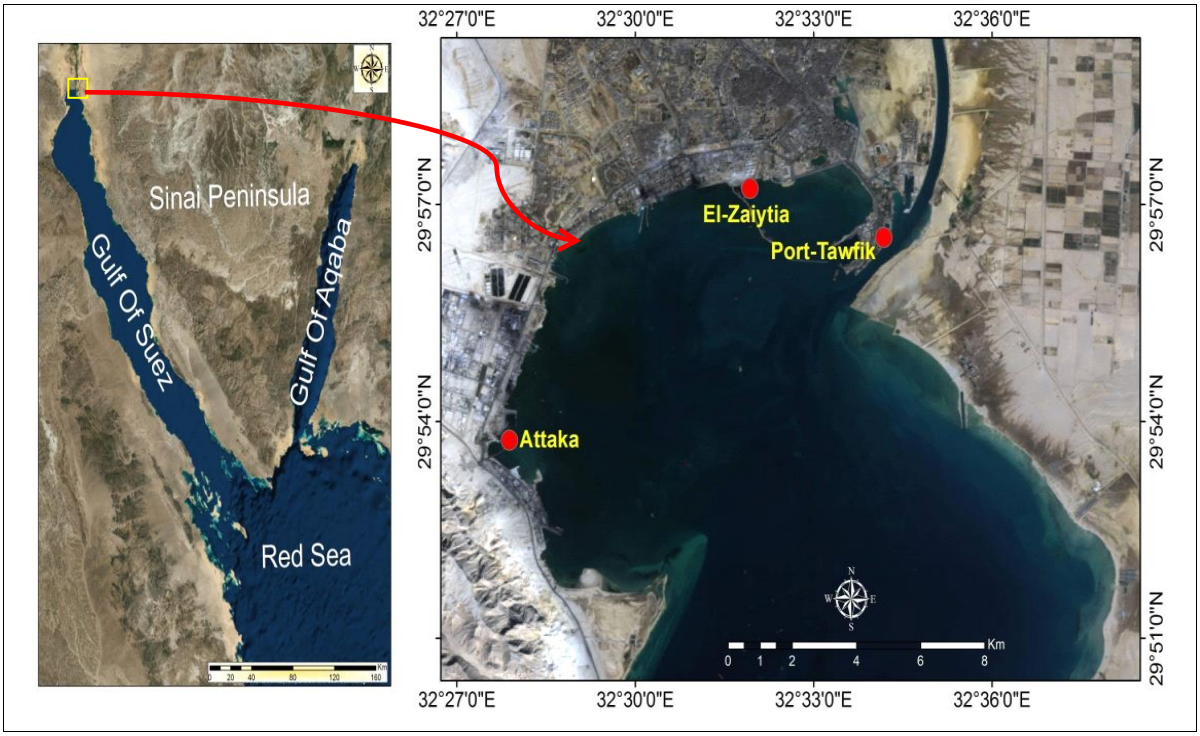

Figure. (1): Map of the study area.

observation of fungi growth every 9 days. Six fungal isolates were tested with anthracene either solid or soluble in ethanol. Flasks were incubated after at (27^{o}C) for (2 weeks). Blanks were prepared for each treatment without fungal inoculation.

Extraction and Quantification of Total Petroleum Hydrocarbons (TPHs) and polyaromatic hydrocarbos (PAHs)

Spectrofluorometric analysis

After inoculation at fixed interval all samples of each substrate were extracted three times with a 60 ml of dichloromethane in a separating funnel. Sample extracts were combined and concentrated by rotary evaporation to 5 ml. Finally, samples were concentrated under a gentle stream of pure nitrogen to a final volume of 2 ml. (Parsons *et al.*, 1985).

Followed by cleanup of samples according to R.O.P.M.E., (1983). Then analyzed and measure the Total Petroleum Hydrocarbons contents by Digital Spectrofluorometer (Sequoi- turner corporation, made in USA.) model 450 with NB 360 for excitation filter and SC 415 for emission filter. And analyzed by capillary gas chromatograph (CGC) for identify the fractions of PAHs according to the standard used.

CGC analysis

After Spectrofluoromtric analysis the extract samples were injected in gas chromatograph analyzer model Hewlett Packed 5890 series II GC gas chromatograph equipped with a flame ionization detector (FID). Chromatographic conditions was capillary column HP-1; 100% dimethyl polysiloxane (30m length 0.32 mm 0.17µm film thickness).The oven temperature was programmed from 60-290°C, changing at a rate of 3 °C /min. followed by 3min hold at 300°C. The carrier gas was nitrogen flowing at 1.2 ml/min. Detector temperature at 300 °C. Mixed Standard solution of PAHs 16 fraction contains Naphthalene (two rings compound), Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene (three rings compounds), Pyrene, Benzo (a) anthracene, Chrysene, Benzo (b) fluoranthcene, Benzo (k) fluoranthcene, Benzo (a) pyrene,(four rings compounds) Indeno (1,2,3cd)pyrene, Dibenzo (a,h) anthracene,and Benzo (ghi) perylene (five rings compounds) . Was prepared at different concentrations to set up replica of calibration curves at 20, 40, 60, and 80 ppm., the volume of injection is 3 µL with splitless (US EPA 610 Method)

RESULT

Water characteristics of the investigated area

Table (1) shows physical; chemical and nutrient salts parameters were measured in the bottom water samples collected from Port-Tawfik ،El-Zaiytia and Attaka locations of the Gulf of Suez during winter season (2016). It is clear that Port-Tawfik location recorded the higher mean levels of Dissolved oxygen reach to 9.07 mgO_2l^{-1} and nutrient salts (nitrite; nitrate and ammonia) reach to 0.803 $\mu moll^{-1}$; 36.68 $\mu moll^{-1}$, and 25.36 µmol/L respectively. Attaka location recorded higher mean levels of temperature reached to 18.6 ^{O}C and pH 8.13. This is in addition to record the TPHs in water reached to higher levels at Attaka in water and higher level in sediments samples at El-Ziaytia. The significant calculated N: P ratios were 62.8: 2.18; 8.9:2.57 and 8.7:1.52 at Port-Tawfik; El-Ziaytia and Attaka respectively.

Table (1): Field analysis of water quality of water samples collected from gulf of Suez during winter 2016.

Parameter / Station	Temp^{0}C	S $\%_0$	pH	DO mgO_2l^{-1}	BOD mgO_2l^{-1}	NO_2-N $\mu moll^{-1}$-	NO_3- N $\mu moll^{-1}$	NH_3- N $\mu moll^{-1}$	PO_4- N $\mu moll^{-1}$
Porttawfik	18.10	40.30	8.12	9.07	3.25	0.803	36.68	25.36	2.184
El-Ziaytia	18.35	40.45	8.10	7.99	2.99	0.313	3.502	5.171	2.578
Attaka	18.60	40.15	8.13	8.89	4.40	0.340	4.529	3.848	1.552

Isolation; identification and screening of fungal species

In the present study, fungi isolated from the most petroleum hydrocarbons-polluted areas of Gulf of Suez recorded 17 fungal isolates in response to the hydrocarbons compounds such as accommodated fractions of crude oil (ACF)[1]; water soluble fraction (WSF)[2] and Anthracene[3] in the broth media and carried out the ability of the biodegradation by fungal species in the laboratory tests as follow as table 2.

Biodegradation

Using ACF as a sole source of carbon

A. *flavus* has the most effective ability to degrade ACF during 35 days reaching 98 .78 % than other fungal positive to degrade ACF. Fig. (2) shows the percentages of degradation and residuals of total petroleum hydrocarbons in ACF during the fixed intervals by specrtoflurometric analysis. It is clear that the rate of degradation is slow and low about 22.4% in the first interval after 17 days, in the second interval the level after 26 days is accelerated reached to about 68 .1% and the third interval after 35 days the degradation level reach to semifinal about 98.8 % removal and reduced of TPH in ACF components. Gas chromatographic analysis shows in Fig (3) that all $\sum_{16}$ fractions of PAHs aromatic rings have been reduced more than 99% during all the intervals.

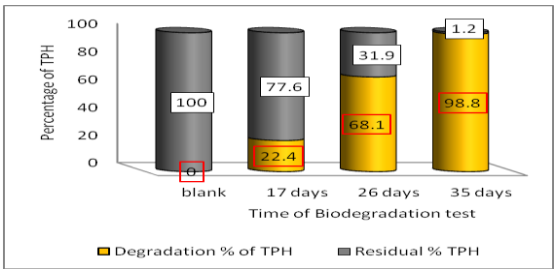

Figure (2): The percentage of fungal degradation (*Aspergillus flavus*) and residual of TPH during the time test of biodegradation.

Table (2): The recorded fungal species from bottom water and sediment samples collected from three stations during winter season (2016).

Station	Sample type	Species
Port-tawfik	Bottom water	*Trichoderma harzianum* [2] *Cladosporium* sp. [3]
	Sediment	*Penicillium chrysogenium* [1,2] *Penicillium* sp. [2]
El-Zaiytia	Bottom water	*Trichoderma viride* [1,2] *Drechslera* sp. [3]
	Sediment	*Apergillus niger* [2] *Cladosporium* sp. [2] *Fusarium* sp. [2]
Attaka	Bottom water	*Cladosporium* sp. [3] *Alternaria alternate* [2]
	Sediment	*Cladosporium* sp. [3] Yeast [2] *Trichoderma* sp. [3] *Aspergillus terrus* [1,2] *Drechslera* sp. [1,2] *Aspergillus flavus* [1,2]

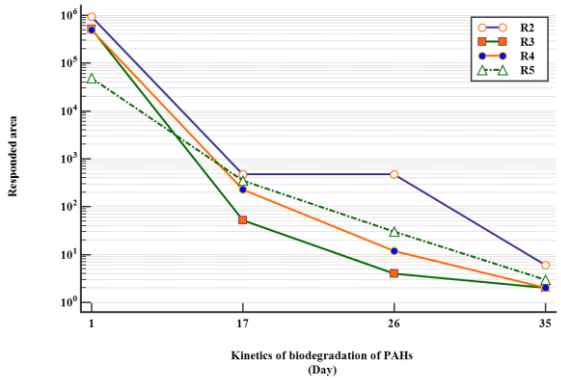

Figure. (3): The reduced of response area of PAHs fractions (two, three, four ,and five rings) by capillary gas chromatograph analysis during all the intervals of test biodegradation of ACF by *A.flavus*.

Using WSF as a sole source of carbon

The efficiency percentage of some fungal isolates on WSF was detected and showed in Table (3) during biodegradation test. The results indicated that; five fungal isolates have the ability to degrade WSF with the efficiency reaching (95.90, 97.45, 96.02, 97.72 and 98.53 %) *Drechslera* sp ،*A .terrus* ،*A .flavus* ،*T .viride* and *P .Chrysogeniu* respectively by Spectrofluorometric analysis. Also, Fig. (4) show the reduced amount of the response area of $\sum_{16}$PAHs fractions by GC analysis of WSF through biodegradation test by some fungal isolates.

Table (3): The biodegradation percentages of total petroleum hydrocarbons of WSF by different fungal species after 14 and 21 days by using spectroflourometric analysis.

Species	Time (Day)	
	14	21
Drechslera sp.	66.21 %	95.90 %
A. terrus	90 %	97.45 %
A. flavus	94.45 %	96.02 %
T. viride	97.16 %	97.72 %
P. chrysogenium	98.50 %	98.53 %

Using anthracene as a sole source of carbon

Efficiency of some fungal species isolated from bottom water and sediment to degrade anthracene as a salt and soluble in alcohol) ethanol) presented in Table (4).The results clarify that *Cladosporium* sp. isolated from water sample collected from Port-tawfik has the most effective ability to degrade anthracene as solid reaching up to56.08.

Table (4): The biodegradation percentages of anthracene (as solid or soluble in alcohol) by different fungal species after 7 and 14 days by using spectroflourometric analysis.

Species	Anthracene solid	Anthracene soluble in ethanol (alcohol)
*Cladosporium*sp.'	56.08	8.05
Trichoderma sp.	43.6	16.88
Drechslera sp.	28.6	15.4
*Cladosporium*sp.'	0	12.94

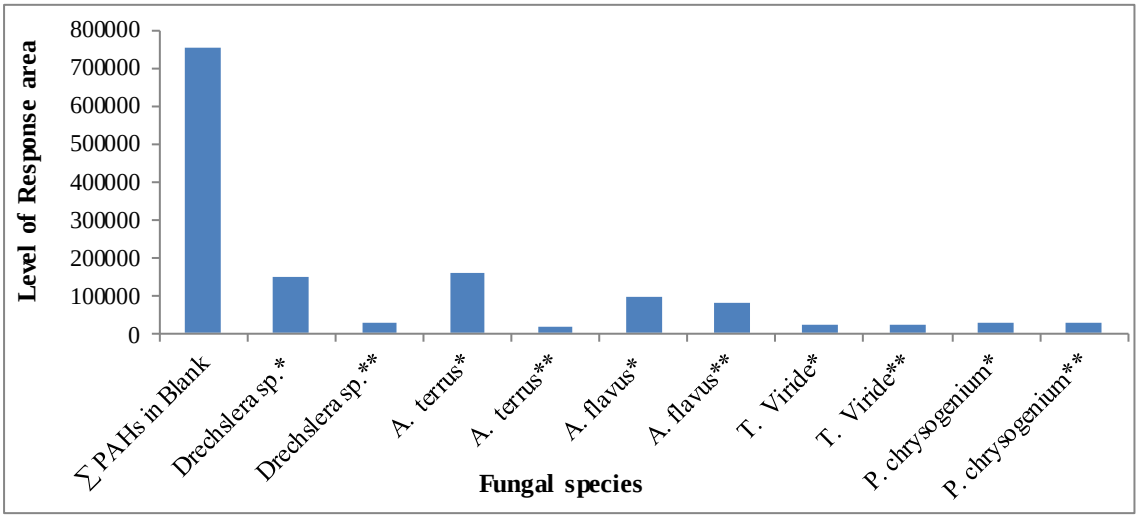

Figure (4): The levels of reduced response area of ∑PAHs by capillary gas chromatographic analysis during the biodegradation of WSF by different fungal species after day 14* and day 21**.

DISCUSSION

This study show the biodegradation of petroleum hydrocarbons using locally isolated fungi isolated from hydrocarbons polluted area in Suez Bay. Environmental conditions and nutrient salts were measured in the field to compare availability of conditions to the biodegradation process through their consumption hydrocarbons as a carbon source. Glaser, 1991 stated that the total nitrogen to total phosphorous ratio N: P nearly 7.6:2.8 is suitable for biodegradation and the present result find that El-Zaiytia location is approach to the suitable condition it reach to 8.9 :2.57. Environmental factors like temperature and pH were considered as important factors as they have a significant effect on the rate of microbial degradation (Tripathi and Srivastava, 2011). So, the field data analysis gives a knowledge and prediction about the variability in physical and chemical parameter may relate to the biological process go ahead or delay.

In vitro conditions, it is detected the ability of 17 fungal species isolated from crude oil-contaminated water and sediments, to degrade ACF, WSF and anthracene. Only five fungal isolates were able to grow on ACF, which identified as *A. flavus*; *A. terrus*; *Drechslera* sp; *P. chrysogenium* and *T. viride*. *A. flavus* isolate was the most effective fungal species which degrade ACF 98.79% after 5 weeks of incubation. This results matching with other studies like Andrea *et al.,* (2001), Obire *et al.,* (2008), Al-Jawhari (2014) and Ameen *et al.,* (2016). They recorded that *Aspergillus* and *Penicillium* species were the most efficient genera in degrading of hydrocarbons.

On the other hand, the results showed that the same five fungal species able to grow and degrade WSF after 10 days from inoculation. *P. chrysogenium*, *T. viride* and *A. terrus* were the most potent to degrade WSF, the efficiency percentage reached to (98.53; 97.72 and 97.45%, respectively) after 21 days. These results supported by other investigators such as Sutherland, (2004), Mtui (2012) and Dessouki *et al.,* (2013).

Three fungal genera identified as (*Cladosporium* sp.; *Trichoderma* sp. and *Drechslera* sp.) were able to degrade anthracene either as a salt and or soluble in ethanol. *Cladosporium* sp. was the most efficient isolate. It was isolated from bottom water in Port-Tawfik Port and from sediment in Attaka Fishing Port, respectively. Other studies found that there are other genera able to degrade anthracene other than *Cladosporium*, like *Phanerochaete chrysogenium* Hammel *et al.,* (1986), *Bjerkandera* sp. Gramss *et al.,* (1999), and *Cunninghamella echinulata* El-Morsy, (2012).

It can be concluded that many fungi metabolize aromatic hydrocarbons with enzymes that include lignin peroxidase, manganese peroxidase, laccase, cytochrome P450, and epoxide hydrolase. The greater capacity to remove crude oil due to the adaptation of these fungi to the pollutant composition, as well as to the enzymatic systems of the fungi (Mance *et al.,* 2007). The enzymatic system affected by many factors as pH , temperature, chemical structure of compounds, pathway of enzyme due to metabolite, and the type of species. So, the culture of fungi that degrade polycyclic aromatic hydrocarbons may be useful for bioremediation of polluted sediments and waters. It is consider the best because their metabolite of degradation is less toxic than the parent molecule (Cerniglia and Sutherland, 2010).

REFERENCE

AI-JAWHARI, I.F. 2014. Ability of some soil fungi in biodegradation of petroleum hydrocarbon. J. of Appl. and Environ.Microbiol, 2 (2), 46-52.

AMEEN, F., M. MOSLEM, S. HADI AND A. AL-SABRI. 2016**.** Biodegradation of Diesel Fuel Hydrocarbons by Mangrove Fungi From Red SeaCoast of Saudi Arabia. Saudi J. Biolog. Sci., 23 (2), 211-218.

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). 2005**.** Standard Methods For The Examination of Water and Wastewater, 21th Edition, Washington DC, USA.

ANDREA, R.C., A.A. TANIA AND R.D. LUCIA. 2001**.** Biodegradation of Polycyclic Aromatic Hydrocarbons by Soil Fungi. Braz. J. Microbiol., 32(4), 124-129.

ANDERSON, J.W., J.M. NEFF, B.A. COX, H.E. TANTAN AND G.M. HIGHTOWER. 1974**.** Characteristics of Dispersion and Water-Soluble Extracts of Crustacean and Fish. Marin. Biol., 27, 75-88.

BALAJI,V., P. ARULAZHAGAN, P. EBENEZER-.2014. Enzymatic Bioremediation of Polyaromatic Hydrocarbons by Fungal Consortia Enriched from Petroleum Contaminated Soil and Oil Seeds. J. Environ .Biol. 35 (3), 521.

BOOTH, C. 1971. The Genus Fusarium. 2nd ed., Common Wealth Mycological Istitute, Kew, Surrey, U.K.

CERNIGLIA, C.E. AND J.B. SUTHERLAND. 2010. Degradation of Polycyclic Aromatic Hydrocarbons by fungi .Handbook of Hydrocarbon and Lipid Microbiology, 2079-2110.

CHIKERE, C., B. AND C.C.AZUBUIKE. 2014. Characterization of Hydrocarbon Utilizing Fungi from Hydrocarbon Polluted Sediments and Water. Nig. J. Biotech., 22, 49-54.

DA SILVA, M.E., J.D. ESPOSITO, E.D. MOODY, V.P. CANHOS AND C.E. CERNIGLIA, 2004. Metabolism of Aromatic Hydrocarbons by the Fil-amentous Fungus *Cyclothyrium* sp. Chem-osphere,57,943-952.

DESSOUKI, A.A., T.M. ABDEL-RASSOL N.S. SHWTAR, H.M. TANTAWY AND N.E. SALEH. 2013. Pathological Effects of Water Soluble Fraction of Burned Motor Oil in *Tilapia zillii* and *Mugil cephalus* Through Bioremediation Process. Middle-East J. Scien. Research, 17 (10), 1386-1395.

ELLIS, M.B. 1971**.** Dematiaceaous Hyphomycetes. 2nd ed., Common Wealth Mycological Institute, Kew, Surrey, England.

EL-MORSY, E.M. 2012**.** Bioremediation of Anthracene, Phenanthrene and Phenol by

Cunninghamella echinulate. Sci. J. for Damietta Faculty of Science, 1, 65-75.

FETZER, J. C. 2000. The Chemistry and Analysis of the Large Polycyclic Aromatic Hydrocarbons. Polycyclic Aromatic Compounds (New York: Wiley) 27, 143.

GLASER, J.A. 1991. Nutrient-Enhanced Bioremediation of Oil-Contaminated Shoreline: The Valdez Experience. in: Hinchee RE & Olfenbuttel RF (Eds) on Site Bioreclamation, Butterworth-Heinemann, Stoneham, MA, 336-384.

GLASER, J. A. 1991. Nutrient-enhanced Bioremediation of Oil-contaminated Shoreline: The Valdez Experience. In: Hinchee RE & Olfenbuttel RF (Eds) on Site Bioreclamation, Butterworth-Heinemann, Stoneham, MA, 336-384**.**

GRAMSS, G., K. VOIGT AND B. KIRSCHE. 1999. Degradation of Polycyclic Aromatic Hydrocarbons with Three to Seven Aromatic Rings by Higher fungi in Sterile and Unsterile soils. J. of Biod., 10 (1), 51-62.

GUTNICK, D.L. AND E. ROSENBERG. 1977. Oil Tankers And Pollution: A Microbiological Approach. Annual Review of Microbiology, 31, 379–396.

HAMMEL, K.E., B. KALYANARAMAN, AND T.K.KIRK.1986. Oxidation of Polycyclic Aromatic Hydrocarbons and Dibenzo[P]Dioxins By Phane-rochaete Chrysosporium Ligninase. J. Biol. And Chemi., 261, 16.948—16952.

MANCE-LOPEZ, M.E., M.T. RODRIGUEZ, E. RIOS-LEAL, F. ESPARZA-CARCIA, B. CHAVEZ-GOMMEZ, R. RODRIGUEZ-VAZQUES AND J. BARRERA-CORTES. 2007. Fungi and Bacteria Isolated from Two Highly Polluted Soils for Hydrocarbon Degradation. J. of Acta Chimica Slovenica, 54, 201-209.

MESHAL, A.H., 1967. A physical Study of the Water Pollution in the Suez Bay (Hydrography of Suez Bay). M. Sc. Thesis, Cairo University, Egypt, 254 pp.

MESSIAS, J.M., DA COSTA, B.Z., DE LIMA, V.M., DEKKER, R.F., REZENDE, M.I., KRIEGER, N., BARBOSA, A.M., 2009**.** Screening *Botryosphaeria* species for lipases: Production of Lipase by *Botryosphaeria ribis* EC-01 Grown on Soybean Oil and other Carbon Sources. Enzyme Microb. Technol. 45 (6), 426–431.

MTUI, G.Y. 2012. Lignocellulolytic Enzymes from Tropical Fungi: Types, Substrates and Applications: Scientific Res Essays., 7(15). 1544-1555.

NEFF, J. M.1979**.** Polycyclic Aromatic Hydrocarbons in The Aquatic Environment: Sources, Fate and Biological Effects. Appl. Sci.Publisher Ltd, London, UK, P. 262. OBIRE, O. AND F.O. AMUSAN. 2003**.** The Environmental Impact Of Oilfield Formation Water on A Freshwater Stream in Nigeria. J. Appl Sci. Environ. Mgt. 7, 61-66.

OBIRE, O. AND S.A. WEMEDO. 1996**.** The Effect of Oil Field Wastewater on the Microbial Population of A Soil in Nigeria. Niger Delta Biologia 1(1), 77-85.

OBIRE, O., R. RAMESH, A. PUTHETI, A. DICK AND R. 18-OKIGBO. 2008**.** Biotechnology Influence For The Production of Ethyl Alcohol Ethanol & from Waste Fruits.E J. Of Sci. Technol. (E- JST), 3(3), 17-32.

PARSONS, T.R., Y. MATIA, AND C.M. LALLI. 1984 Determination of Chlorophylls and Total Carotenoids: Spectrophotometric Method, A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamun Press, New York, 101-104.

PARSONS, T.R., Y. MATIA AND G.M. MALLI. 1985**.** Determination Of Petroleum Hydrocarbons. A Manual of Chemical and Biological Method for Seawater Analysis. Pergamun Press, Oxford., 173

PITT, J.I. 1979. The Genus Penicillium and Its Teleomorphic States Eupenicillium and Talaromyces. Academic Press, London.

PLICE, M.J. 1948. Some Effects of Crude Petroleum on Soil Fertility. Soil Science Society Proceedings, 13, 413-416.

R.O.P.M.E. 1983**.** Manual of Oceanographic observation and Pollution Analysis Methods. Al-Safat, Kuwait.

SMITH, N.R. AND V.T. DAWSON 1944**.** The Bacteriostatic Action of Rose-Bengal in Media Used for the Plate Count of Soil Fungi. Soil Sci., 58, 467-471.

SUTHERLAND, J.B. 2004. Degradation of Hydrocarbons by Yeasts and Filamentous Fungi. in: Fungi Biotechnology in Agricultural, Food and Environmental Applications. Arora, D., K., (ed.). Marcel Dekker Inc.

SUTHERLAND, J.B., F. RAFII, A.A. KHAN, AND C. E CERNIGLIA. 1995. Mechanism of Polycyclic Aromatic Hydrocarbon Degradation. in: Microbial Transformation and Degradation of Toxic Organic Chemicals. Eds., L.Y. Young and C.E. Cerniglia. Wiley-Liss. New York, N.Y., 269-306.

TRIPATHI, A.D. AND S.K. SRIVASTAVA, 2011**.** Novel Approach for Optimization of Fermentative Condition for Polyhydroxybutyrate (PHB) Production by *Alcaligenes sp*. Using taguchi (DOE) Methodology. J. of Biotechno., 10, 7219-7224.

US EPA 610: Appendix A to Part 136 Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, Method 610—Polynuclear Aromatic Hydrocarbons.

التحلل الحيوي لبعض الهيدروكربونات البترولية بالفطريات المعزولة من خليج السويس

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الملخص العربى

تعد الهيدروكربونات البترولية واحدة من الملوثات الرئيسية التى تؤثر مباشرة فى خليج السويس بسبب ما ينتج و يلقى من المنتجات البترولية كما فى الانبعاثات من احتراق الوقود و من انشطة الشحن و التكرير. هناك عدة طرق لمعالجة الملوثات الهيدروكربونية مثل الطرق الفيزيائية والميكانيكية والكيميائية والبيولوجية. استخدم الجزء المائى المتشرب (ACF) و الجزء الذائب (WSF) من خام البترول و الانثراثين بشكل منفصل كمصدر وحيد للكربون مع الوسط المغذى السائل فى اختبار التحلل الحيوى . تم عزل 17 نوعا فطريا من المياه والرواسب من ثلاث محطات مختارة (بورتوفيق والزيتية والاتكة) ثم تم فحصها مع كل ركيزة. التحليل الغازكروماتوجراف الشعرى استخدم لتنميط الجانب الكيميائى لكل ركيزة بعد التلقيح. اظهرت النتائج أن *Aspergillus flavus* كان الأكثر فاعلية لتدهور ACF ليصل إلى (98.79٪). و في حالة WSF كانت العزلة *Penicillium chrysogenium* أعلى نسبة تدهور (98.53٪). و تدهور الأنثراسين بعد أسبوعين سجل (56.08٪) باستخدام *Cladosporium sp*. في الختام ، يحتوي خليج السويس على العديد من الأنواع الفطرية الواعدة التي يمكن استخدامها في التحلل البيولوجي للهيدروكربونات النفطية كبدائل حفظ في النظام البيئي البحري.

الكلمات المفتاحية: التحلل الحيوي الفطري ،الهيدروكربونات البترولية، أنثراسين ،كروماتوجرافيا الغاز الشعري ، خليج السويس.