Mycoremediation of Benzene, Toluene, Ethyl benzene and Xylene (BTEX) Compounds by Fungi Isolated from Hydrocarbon-contaminated Soil

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Abstract: Benzene, toluene, ethyl benzene and xylene(BTEX)are the most commonly cited monoaromatic compounds in environmental pollution in recent years due to their high solubility and toxicity in ground water and soils. This studywas conducted in order to isolate and enumerate fungi in soils heavily polluted with petroleum hydrocarbons, and to select those with BTEX degrading abilities. Screening of the fungi isolated on Mineral Salt Media (MSM) supplemented with BTEX showed *Aspergillus terreus* DMW-5 and *Gliocladium* spp. had the highest colony diameter while *Penicillium* spp. has the lowest colony diameter. The highest BTEX weight loss and increase in spore size was also observed with *Aspergillus terreus* DMW-5 and *Gliocladium* spp. which degraded 240mg/mL BTEXand 220mg/mL BTEX while the lowest was with *Aspergillus flavus* which degraded 190mg/mL BTEX. The hydrocarbon utilizing fungal (HUF) counts revealed *Aspergillus terreus* DMW-5 and *Gliocladium* spp. as having the highest number of spores (8.2×10^7 and6.3×10^7 spores/mL) and *Aspergillus flavus* with the lowest number of spores (1.2×10^7 spores/mL). Thus, *Gliocladium* spp.and *Aspergillus terreus* DMW 5 are potential candidates for the mycoremediation studies of BTEX.
Key words: BTEX, Mycoremediation, Fungi, *Aspergillus*, *Gliocladium*

INTRODUCTION

Benzene, ethyl benzene, toluene and the polymers of xylene are collectively known as BTEX, and are monoaromatic hydrocarbons which are important environmental pollutants (Tobler *et al.,* 2008). These BTEX are groundwater, soil and air pollutants commonly associated with petroleum and petrochemical production (Cohen and Mercer, 1993). BTEX contamination of soil and groundwater is usually related to petroleum leakages from underground storage tanks, manufacturing of solvent-based paints, lacquers and varnishes and the activities of manufactured gas plants (Bushwell, 2001). Significant quantities of these contaminants inevitably get into the environment during the production process. The compounds represent about 80% v/v of the total Volatile Organic Compounds in petrochemical plants (Fatehifar *et al.,* 2008). Such compounds accounted for 59% (w/w) of gasoline pollutants (Barona *et al.,* 2007). They are harmful to the ecosystem, human health and atmosphere (Adam *et al.,* 2001) and are among the major air pollutants due to their malodorous and hazardous properties (Durmusoglu *et al.,* 2010). The compounds readily volatize to the atmosphere and distribute over large regions because of their relatively high vapor pressure (Bushwell, 2001). Their emission in the atmosphere causes different environmental problems such as ground level ozone formation, stratospheric ozone depletion, photo chemical reactions, greenhouse effect (Muhammad *et al.,* 2007) and global warming (Durmusoglu *et al.,* 2010). There are two major steps involved in the bioremediation of aromatic compounds which are activation of the ring and ring cleavage (Kothari *et al.,* 2004). Generally, the aerobic degradation of mono-aromatic compounds follows different metabolic pathways based on the enzyme system present in the microorganisms (Cao *et al.,* 2009). Activation is achieved by the incorporation of molecular oxygen into the aromatic ring leading to dihydroxylation of the aromatic nucleus, and the enzymes responsible for this are oxygenases. Monooxygenases, characteristic of fungi and other eukaryotes, catalyze the incorporation of a single atom of oxygen to form an epoxide which can then undergo hydration to yield transdihydrodiols.

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Dioxygenases catalyze simultaneous incorporation of two atoms of oxygen to form a dihydrodiol. These dioxygenase reactions have been shown to occur in benzene, halogenated benzenes, toluene, para-chlorotoluene, xylenes, biphenyls, naphthalene and anthracene. These dihydrodiols are further oxidized to catechols which are the precursors to ring cleavage. Catechol can be oxidized either via ortho-cleavage pathway which involves cleavage of the bond between carbon atoms of the two hydroxyl groups to yield muconic acid, or via the meta- cleavage pathway which involves cleavage of the bond between a carbon atom with a hydroxyl group and the adjacent carbon atom to yield 2-hydroxymuconic semi aldehyde. These compounds are further degraded to form organic acids which are then utilized by microorganisms for their cell synthesis and energy generation (Kothari *et al.,* 2004). Biodegradation by microorganisms represent one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment (Brar *et al.,* 2006). Fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria (Belli, 2004). Fungi capable of degrading BTEX have been isolated from soil (Yadav and Reddy, 1993; Hardison *et al.,* 1997; Deeb *et al.,* 2001). Because of their aggressive growth, greater biomass production and extensive hyphal growth in the soil, fungi offer potential for biodegradation technology. Filamentous fungi play an important role in degrading petroleum hydrocarbons by producing capable enzymes. Some fungi exude extracellular enzymes which catalyse the digestion of energy sources in their surroundings (Sabah *et al*., 2016).This study was aimed at assessing the potential of fungal isolates from hydrocarbons contaminated soil to degrade BTEX compounds.

MATERIALS AND METHODS

The BTEX hydrocarbons used in this work comprised of a mixture of benzene (99.9% purity, M & B, England), toluene (99.5% purity, BDH, England), ethylbenzene(99% purity, JHD, China),and xylene isomers mixture (99% purity, JHD, China).The extracting solvent, Dichloromethane (DCM) is a product of Merck, and 98% purity. It was further distilled to obtain DCM of higher purity and also of analytical standard.

The mineral salt medium (MSM) used in the experiment consisted of a mixture ofNa_2HPO_4 (2.0g), K_2SO_4 (0.17g), NH_4NO_3 (4.0g), KH_2PO_4 (0.53g), $MgSO_4{\cdot}7H_2O$ (0.5g) and 1.0mL of a trace salt solution per liter of distilled water after Ekundayo *et al.* (2012). A stock solution of trace salt containing $CoCl_2{\bullet}6H_2O$(30mgL-1), $CuCl_2$ (0.15mgL-1), H_3BO_3 (5.7mgL-1), $MnCl_2{\bullet}4H_2O$ (20mgL-1), $Na_2MoO_4{\bullet}2H2O$ (2.5mgL-1), $NiCl_2{\bullet}2H_2O$ (1.5mgL-1), $ZnCl_2$ (2.1mgL-1) (Jian-zhong *et al.,* 2009).

Potential BTEX-degrading fungi were isolated from hydrocarbon contaminated soil collected from Dan-magaji Mechanic Workshop, Zaria, Kaduna state, Nigeria, using the standard methods (Prenafeta-Bold´u *et al.,* 2004). Ten gram of soil sample was transferred to the 100ml of Mineral Salt Medium (MSM) in 250ml of conical flask with 1% (v/v) of BTEX as a carbon source. The flask was incubated at 25^0C on a rotatory shaker at 180 rpm for 14days (Kamal *et al.,* 2017). Then 10ml of culture was transferred to fresh MSM containing 1% (v/v) and incubated for another 7 days. After 2 successive cycles of such enrichment, 1mL of the culture was used to make ten-fold dilution, up to a dilution of 10^{-5}(Prenafeta-Boldu *et al.,* 2001). Spread plate method was used to inoculate 0.1mL aliquot of dilutions 10^{-2} to 10^{-5} onto the surface of SDA. The plates were incubated at 25^0C for 7days (Harley and Prescott, 2002). The prominent fungal colonies were selected and pure culture obtained for further study.

The cultural characteristics of the purified isolates were noted and the microscopic features of the isolates were observed using the wet mount technique (Sharma, 2009). Purified cultures were stored on SDA slants for further characterization. Lactophenol cotton blue stain was used as mordant. The microscopic structures observed were recorded and compared to those stated in previous studies (Aneja, 2005).

Preparation and standardization of fungal spore suspensions

This was carried out following the method of Machido *et al.* (2014). The fungal isolates were grown on SDA slants for 3-5days to obtain heavily sporulated cultures. The spores were scraped gently using a sterile inoculating needle under sterile aseptic conditions. Spores suspension of the isolates were obtained by dispensing 15ml of sterile distilled water containing 0.005% Tween 80 into an agar slant and shaken properly for 15minutes, to wash off the spores. The spore suspensions were diluted with sterile distilled water to obtain concentration of spores/ml. The spores were enumerated by direct counting, using neubauer hemocytometer.

Two milliliters (2mL) of the standardized cell suspensions were inoculated into 5ml of mineral salt medium supplemented with 1% v/v BTEX and 0.1% (v/v) Tween 80 (George- Okafor *et al.,* 2009). Incubation was done at 150rpm ina shaker at ambient temperature for 96 hrs (Nwankwegu*et al.,* 2016).

Screening the isolates for ability to grow on BTEX

The growth assay was used to determine the fungal species resistant to BTEX (AI-Jawhari, 2014). This was conducted by comparing the growth rates of the fungal isolates by measuring their colony diameter on solid media supplemented with BTEX. Test plates were prepared by adding 1% v/v BTEX to mineral salt agar before it solidified; the solution was thoroughly mixed with a sterile magnetic stirrer, right before it was poured into the sterile petri dishes. The media were inoculated with 5mm plugs of fungal mycelia taken from the agar inoculum plate; they were then incubated at 25^0C.Fungal mycelia extension on the plates (colony diameter) was measured after ten (10) days using a measuring tape (AI-Jawhari, 2014).

Assessment of the BTEX-degrading potential of the isolates

A screening test was used to assess the biodegradation ability of each fungal isolate (George- Okafor *et al.,* 2009). One milliliter of a 96h cell suspension (containing approximately 1 x 10^6 spores/mL) of each isolate was inoculated into a Erlenmenyer flask (250mL) containing MSM supplemented with BTEX (1% v/v) while the control flask was left uninoculated. Incubation was done at 25^0C on a rotary shaker at 180rpm for 7days. After the period of incubation, 5mLaliquots were collected from each flask and transferred into test tubes, the residual BTEX was extracted by adding 5ml dichloromethane (DCM) and centrifuged (5000rpm) for 5minutes and the supernatant read at 600nm wavelength using a UV- Visible spectrophotometer after Nwankwegu *et al.* (2016). Weight loss of BTEX due to fungal degradation was calculated after determining the amount of BTEX from a prepared standard using known amounts of BTEX. One milliliter aliquots was also collected from each flask and used to make a 10 fold dilution up to 10^{-3}. This dilution was used to estimate growth of fungi by direct counting using a haemocytometer (Bekada *et al.* 2008; Bekker *et al.* 2009).

Percentage of degradation = $\left[\frac{\text{TBTEX control} - \text{TBTEX treatment}}{\text{TBTEX control}}\right] * 100$(Manal, 2011).

Where TBTEX control represents the total residual BTEX of the control

TBTEX treatment represents the total residual BTEX of each inoculated flask

RESULTS

The enrichment technique resulted in 8 fungal isolates having the ability to grow in Mineral Salts Medium (MSM) with BTEX as sole source of carbon. These fungal isolates includes; *Gliocladium* spp., *Aspergillus flavus, Aspergillus terreus* DMW-3*, Penicillium* spp.*, Aspergillus terreus* DMW-5, *Trichoderma* spp., *Aspergillus terreus*DMW-7and *Aspergillus niger.*

Screening of isolates for BTEX-degrading potential

The ability of the isolated fungal strains to grow solely on BTEX was expressed as diameter of the colony on the solid medium (Table 1). The table shows that all the fungi are resistant to the presence of BTEX. Among the fungi isolate, *Aspergillus terreus* DMW-5 showed the highest resistance to the presence of (BTEX1%), with 7.8 cm diameter of colony after 10 days growth, while the lowest resistance was observed in *Penicillium* spp. (3.2 cm).

Table 1: Growth on BTEX-MSA medium

Codes	Fungi Isolates	Colony diameter (mm)
DMW-1	*Gliocladium* spp.	7.3
DMW-2	*Aspergillus flavus*	5.5
DMW-3	*Aspergillus terreus*	7
DMW-4	*Penicillium* spp.	3.2
DMW-5	*Aspergillus terreus*	7.8
DMW-6	*Trichoderma* spp.	5.6
DMW-7	*Aspergillus terreus*	7.2
DMW-8	*Aspergillus niger*	6.9

Note: DMW= Dan-magaji Mechanic Workshop

Biodegradation of BTEX compounds by fungi isolated from petroleum-contaminated soils.

Table 2 and figure 1 showed the amount of BTEX reduced by each isolate. The quantitative approach illustrates the total biodegradation loss of the BTEX compounds. Biodegradation loss varied between (190 and 240mg/mL). All the isolates were able to degrade ≥50% of the BTEX hydrocarbons after 7 days but the highest reduction was observed in *Aspergillus terreus*DMW-5 (63%) and *Gliocladium* spp.(58%), while the lowest reduction of the compounds was observed in *Aspergillus flavus* (50%).

Table 2: Percentage reduction of BTEX compounds by the fungi isolated using spectrophotometric method

Codes	Isolate	Absorbance	Concentration (mg/mL)	Weight loss (mg/mL)	Percentage weight loss (%)
DMW 1	*Gliocladium* spp.	0.053	160	220	58
DMW 2	*Aspergillus flavus*	0.062	190	190	50
DMW 3	*Aspergillus terreus*	0.056	170	210	55
DMW 4	*Penicillium* spp.	0.058	180	200	53
DMW 5	*Aspergillus terreus*	0.048	140	240	63
DMW 6	*Trichoderma* spp.	0.057	170	210	55
DMW 7	*Aspergillus terreus*	0.056	170	210	55
DMW 8	*Aspergillus niger*	0.057	170	210	55

Note: Control absorbance and concentration is 0.098 and 380 mg/mL respectively.

The Degradation test was confirmed by the rate of growth observed among the fungal isolates. The best performing isolates are *Aspergillus terreus*DMW-5 and *Gliocladium* spp. has 8.2 x 10^7 and6.3 x 10^7spores/mL respectively, while *Aspergillus flavus* was the least performing isolate with 1.2 x 10^7spores/mL, while It was observed that the fungi were utilizing BTEX compounds for growth as evidenced by the increasing hydrocarbon utilizing fungal counts (table 3).

Table 3: Hydrocarbon Utilizing Fungal (HUF) counts of fungi during biodegradation experiment

Codes	Fungi isolates	Initial spore count (spore/mL)	Final spore count (spore/mL)	Increase in spore count (spore/mL)
DMW-1	*Gliocladium* spp.	1.2 x 10^6	6.3 x 10^7	6.2 x 10^7
DMW-2	*Aspergillus flavus*	1.2 x 10^6	2.0 x 10^7	1.2 x 10^7
DMW-3	*Aspergillus terreus*	1.3 x 10^6	4.2 x 10^7	3.9 x 10^7
DMW-4	*Penicillium* spp.	1.1 x 10^6	2.4 x 10^7	2.0 x 10^7
DMW-5	*Aspergillus terreus*	1.3 x 10^6	8.2 x 10^7	7.9 x 10^7
DMW-6	*Trichoderma* spp.	1.2 x 10^6	5.8 x 10^7	5.7 x 10^7
DMW-7	*Aspergillus terreus*	1.3 x 10^6	6.1 x 10^7	6.0 x 10^7
DMW-8	*Aspergillus niger*	1.4 x 10^6	5.2 x 10^7	5.1 x 10^7

DISCUSSION

The fungi isolated are known hydrocarbon degraders, and have been the subject of many studies on bioremediation (George-Okafor *et al*., 2009; Ekundayo *et al*., 2012; Machido *et al*., 2014). The measurement is rapid and simple being used by several researchers (Sakineh *et al.*, 2012; AI-Jawhari, 2014). All the isolates screened showed evidence of hydrocarbon degradation; with *Aspergillus terreus*DMW-5 and *Gliocladium* spp. having the highest colony diameter while *Penicillium* spp. had the lowest colony diameter. The growth rate is directly proportional to the rate of breakdown of hydrocarbons. This is similar to the findings of Sakineh *et al*. (2012) in which *Penicillium* spp. showed poor growth on minimal medium containing crude oil and was considered sensitive to oil or was not able to use oil as carbon source. There were three strains of *Aspergillusterreus*DMW-5 but they performed in varying capacities.

The percentage of BTEX lost by the isolates screened varied between 50% to 63%. The isolates were able to degrade more than 50% of the BTEX hydrocarbons after 7 days, but the highest loss was observed with *Aspergillus terreus*DMW-5 and *Gliocladium* spp. This could likely be as a result of more active enzymatic capabilities of these two strains. Fungi degrade hydrocarbons either through the use of lignolytic or non lignolytic enzymes (Raji, 2016), and it is possible that *Aspergillus terreus* DMW-5 and *Gliocladium* spp. Both expressed the genes coding for these enzymes more than the other isolates. Fungi belonging to the genus, *Aspergillus* have diverse metabolic capabilities ranging from degradation of hydrocarbons to biosorption of heavy metals, and they have been associated with soils contaminated with xenobiotics (Buswell, 2001; Chikere and Azubuike, 2014; Nwankwegu *et al*., 2016; Abdullahi, 2017). It has been reported that *Aspergillus* species had the ability for relatively better spore formation than other molds (Abdullahi, 2017). This might substantially improve their ability to utilize organic compounds. Even though the highest spore count was observed in the strain of *Aspergillus terreus*DMW-5(8.2 x 10^7 spore/mL) selected for the biodegradation studies, the increase in the spore count varied slightly between 1.2 x 10^7 to 7.9 x 10^7(spore/mL).

This shows that the numbers of fungal spores are relatively similar with functional proficiencies. This point the comparable spore counts of the fungi are evidence that they all thrived in the presence of the hydrocarbons. This goes to show that studies on the abundance of functional genes for the hydrocarbon degradation will provide more comprehensive information on the metabolic abilities of the isolates (Higashioka *et al*., 2009; Ding *et al*., 2010; Korotkevych *et al*., 2011). There are several reports on reported that *Gliocladium* spp., *Aspergillus flavus*, *Aspergillus terreus, Penicillium* spp., *Trichoderma* spp. and *Aspergillus niger* isolated from petroleum contaminated soils were capable of utilizing crude oil components and polycyclic aromatic compounds as carbon source.

(Sakineh *et al*., 2012; AI-Jawhari, 2014; Chikere and Azubuike, 2014; Ekundayo *et al.,* 2014 and Nwankwegu *et al.,* 2016)

CONCLUSION

This study revealed that *Aspergillus terreus* DMW-5and *Gliocladium* spp. have the highest colony diameter while *Penicillium* spp. as the lowest colony diameter. It was observed that *Aspergillus terreus* had the highest ability to degrade BTEX (63%), followed by *Gliocladium* spp.(58%).It also revealed the increase in the spore count between 1.2 x 10^7 - 7.9 x 10^6 (spore/mL). The highest increase in spore count was observed with *Aspergillus terreus* DMW-5and *Gliocladium* spp. (7.9 x 10^7 and6.2 x 10^7 CFU/mL), while the lowest is with *Aspergillus flavus* (1.2 x 10^7 CFU/mL).Nevertheless, all the isolates showed high effectiveness (< 50%) and can be employed in mycoremediation of BTEX

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REFERENCES

Abdullahi, M.A. (2017). Removal of heavy metals from refinery effluent using sugarcane bagasse inoculated with *Aspergillus flavus* and *Trichophyton* species. Master's Thesis, Department of Microbiology, Faculty of Life Science, Ahmadu Bello University, Zaria, Nigeria.

Adam, J. H., Consatable, J. V. H., Guenther, A. B. and Zimmerman, P. (2001). An estimate of natural volatile organic compound emissions from vegetation since the last glacial maximum. *Chemosphere - Global Change Science*, **3**:73–91.

AI-Jawhari, I. F. H. (2014). "Ability of Some Soil Fungi in Biodegradation of Petroleum Hydrocarbon." *Journal of Applied & Environmental Microbiology,* **2**(2):46-52.

Aneja, K. R. (2005). Experiments in Microbiology, *Plant pathology and Biotechnology*. 4th edn. New Delhi: New Age Publication, pp. 606.

Barona, A., Elias, A., Arias, R., Acha, E. and Cano, I. (2007). Desorption and biofiltration for the treatment of residual organic gases evolved in soil decontamination processes. *Chemical Engineering & Technology*, **30**(1):499–505.

Bekada, A. M. A., Benakriche B., Hamadi K. and Bensoltane A. (2008). Modelling of effects of water acidity, pH and Temperature on the growth rate of *Muco rracemosus* isolated from Camembert Cheese. *World Journal of Agricultural Science,* **4**:790-794.

Bekker, T. F., Kaiser C. and Labuschage N. (2009). The antifungal activity of potassium silicate and the role of pH against selected plant pathogenic fungi in vitro. *South African Journal of Plant Soil,* **26**(1):55-57.

Buswell, J. A. (2001). Fungal biodegradation of chlorinated monoaromatics and BTEX compounds, in: G.M. Gadd (Ed.), Fungi in Bioremediation. Cambridge, Cambridge University Press, pp. 113–35.

Cao, B., Loh, K. and Nagarajan, K. (2009). Biodegradation of Aromatic Compounds; Current Status and Opportunities for Biomolecular Approaches. *Journal of Applied Microbiology and Biotechnology,* **9:**(2)21-92.

Chikere, C. B. and Azubuike, C. C. (2014).Characterization of hydrocarbon utilizing fungi from hydrocarbon polluted sediments and water. *Nig. J. Biotech.,* **27**:49–54.

Cohen, R. M. and Mercer, J. W. (1993).DNAPL Site Evaluation, Boca Raton, FL, C. K. Smoley.

Deeb, R. A., Hu, H. Y., Hanson, J. R., Scow, K. M. and Alvarez, C. L. (2001). Substrate interactions in BTEX and MTBE mixtures by an MTBE-degrading isolate. *Environmental Science and Technology,***35**:312–317.

Ding, G.C., Heuer, H., Zuhlke, S., Spiteller, M., Pronk, G.J., Heister, K., KogelKnabner, I. and Smalla, K. (2010). Soil type-dependent responses to phenanthrene as revealed by determining the diversity and abundance of polycyclic aromatic hydrocarbon ring hydroxylating dioxygenase genes by using a novel PCR detection system. *Applied and EnvironmentalMicrobiology*,**76**(14): 4765-4771.

Durmusoglu, E., Taspinar, F. and Karademir, A. (2010). Health risk assessment of BTEX emissions in the landfill environment. *Journal of Hazardous Materials*, **17**(6):870–877.

Ekundayo, F. O., Olukunle, O. F. and Ekundayo, E. A. (2012). Biodegradation of Bonny light crude oil by locally isolated fungi from oil contaminated soils in Akure, Ondo state. *Malaysian Journal of Microbiology,***8**(1):42-46.

Fatehifar, E., Kahforoshan, D., Khazini, L., Soltanmohammadzadeh, J. S. and Sattar, H. R.(2008). Estimation of VOC emission from wastewater treatment unit in a petrochemical plant using emission factors, in: WSEAS Conferences Cantabria, Spain, Santander.

George-Okafor, U., Tasie, F. and Moutoe-Okafor, F. (2009). Hydrocarbon degradation potentials of indigenous fungal isolates from petroleum contaminated soils. *Journal of Physical and Natural Sciences*, **3**:1–6.

Hardison, L. K., Curry, S. S., Ciuffetti, L. M. and Hyman, M. R. (1997).Metabolism of diethyl ether and cometabolism of methyl *tert*-butyl ether by a filamentous fungus, a *Graphium* sp. *Applied and Environmental Microbiology*.**63**:3059–3067.

Harley, J. P., Prescott, L. M. (2002). Laboratory Excercises in Microbiology.5th edn. New York: MacGraw- Hill, pp. 449.

Higashioka, Y., Kojima, H., Sato, S. and Fukui, M. (2009). Microbial community analysis at crude oil-contaminated soils targeting the 16S ribosomal RNA, xylM, C23O, and bcr genes. *Journal of Applied Microbiology*, **107**:126-135.

Jian-zhong, L. I., Xiang, L. I. U. and Dong, D. E. N. G. (2009). A Study on Anaerobic Biodegradation of BTEX in Soil. National Program Project, PR China (No. 2006CB403400).

Korotkevych, O., Josefiova, J., Praveckova, M., Cajthaml, T., Stavelova, M. And Brennerova, M.V. (2011). Functional adaptation of microbial communities from jet fuel contaminated soil under bioremediation treatment: Simulation of pollutant rebound. *FEMS Microbiology Ecology*,**78**:137-149.

Kothari, V., Panchal, M. and Srivastara, N. (2004). Microbial Degradation of hydrocarbons. Institute of Science, Nirma, India.

Machido, D. A., Yakubu, S. E. and Ezeonuegbu, B. A. (2014). Composition of Fungal Flora in Raw refinery Effluent, Retention Pond and a Treated Effluent Recipient River. *Journal of Applied Science and Environmental Management*, **18**(4):592-596.

Manal, E. A. (2011). Biodegradation of crude oil by bacteria isolated from produced formation water on onshore oil field in Sudan, University of Khourtoum, Sudan.

Muhammad, B. T., Veiga, M. C. and Kennes, C. (2007). Mesophilic and thermophilic biotreatment of BTEX-polluted air in reactors. *Biotechnology and Bioengineering,* **97(**14):23–38.

Nwankwegu, A. S., Edna, I. C. and Nwando, M. O. (2016). Hydrocarbon Degradation Potentials of Fungi Associated with Oil-Contaminated Soil from Selected Mechanic Workshops in Awka, Anambra State, Nigeria. *Frontiers in Environmental Microbiology,***2**(6):38-44.

Prenafeta-Bold´u, F. X., Ballerstedt, H., Gerritse, J. and Grotenhuis, J. T. C. (2004). Bioremediation of BTEX Hydrocarbons: Effect of Soil Inoculation with the Toluene Growing Fungus *Cladophialophora* sp. strain T1. *Biodegradation,* **15:**59–65.

Prenafeta-Bold'u, F. X., Kuhn, A., Dion, M. A.M., Heidrun, A., Johan, W., Van, G. and Jan, A. M. (2001).Isolation and Characterization of Fungi Growing on Volatile Aromatic Hydrocarbons as their Sole Carbon and Energy Source. *Mycology Research Journals.* **105**(4):447-484.

Raji, H. M. (2016). Characterization and molecular diversity of some soil prokaryotes with potentials for polycyclic aromatic hydrocarbon degradation. Department of microbiology, faculty of science, Ahmadu Bello University, Zaria, Nigeria.

Sabah, G., Jatau, E. D. and Whong, C. M. Z. (2016).Assessment of Biodegradation Ability of *Aspergillus niger* Isolated From Mechanic Workshops Soil on Refinery Effluent and Petroleum Hydrocarbons. *International Journal of Scientific and Research Publications,* **6**(3):2250-3153.

Sakineh, L., Gunale1, V. R. and Rajurkar, N. S. (2012).Assessment of petroleum hydrocarbon degradation from soil and tarball by fungi. *Bioscience discovery*, **3**(2):186-192.

Sharma, P. (2009). Manual of Microbiology: Tools and Techniques. 2nd edn. New Delhi: Ane books, pp. 405.

Tobler, N. B., Hofstetter, T. B. and Schwarzenbach, R. (2008). Carbon and Hydrogen Isotope Fractionation during Anaerobic Toluene Oxidation by *Geobacter metallireducens* with Different Fe(III) Phases as Terminal Electron Acceptors. *Environ. Sci. Technol.,***42**:7786-7792.

Yadav, J. S. and Reddy, C. A. (1993).Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium. Applied and Environmental Microbiology*, **59:**756–762.