Waste Engine Oil Degrading Potentials of Indigenous Fungi Isolated From Auto-Mechanic Workshops: Impacts of Heavy Metals (Zn and Pb) Co-Contamination and pH

Mbachu, A.E.*1, Chukwura, E.I.1 and Mbachu, N.A.2
1. Department of Applied Microbiology & Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Nigeria.
2. Department of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.
*Corresponding author; Email: ebelembachu@yahoo.com; Tel: +2348036472525

ABSTRACT
Waste engine oil (WEO) constitutes a potential hazard to humans, animals and vegetation. Studies on the effects of metals on organic pollutant biodegradation demonstrate that metals have the potential to inhibit pollutant biodegradation. Fungi were isolated from soil samples contaminated with WEO using vapour phase transfer method. The ability of the isolates to utilize WEO was assessed using gravimetric method. The impact of Zn and Pb and the effect of pH (5.5, 7.0 and 8.5) on WEO biodegradation by the pure and consortium culture of the isolates were determined. A total of 8 fungal isolates were obtained in this study. 4 that showed high hydrocarbonolastic potentials were confirmed as Candida tropicalis, Rhodosporidium toruloides, Fusarium oxysporium and Aspergillus clavatus using 18S r RNA gene sequence. C. tropicalis and A. clavatus exhibited the highest extent of biodegradation of WEO and were therefore selected for further studies. Although there was significant (P<0.05) increase in inhibition of WEO degradation at high concentration of the heavy metals with increase in pH, low concentration of the metals stimulated the degradation of used engine oil. Highest stimulation of 10.1% and 14.2% was recorded in the presence of 1.0 mg/L Zn and Pb at pH 5.5, with the consortium culture and A. clavatus, respectively. The results showed that the pure and consortium culture of the isolates (C. tropicalis and A. clavatus) have promising potential for effective bioremediation of waste engine oil polluted soil co-contaminated with low levels of Zn, and Pb at pH 5.5.

Keywords: Waste engine oil, degradation, heavy metals, co-contamination, pH.

INTRODUCTION
Indiscriminate disposal of engine oil into gutters, water drains, open vacant plots and farms is a common practice in Nigeria especially by motor mechanics [1]. Heavy metals such as Vanadium, Lead, Aluminium, Nickel and Iron usually below detectable limits in unused lubricating oil have been reported to give high values (ppm) in used oil [2]. These metals
may be retained in soils in the form of oxides, hydroxides, carbonates, exchangeable cations, and/or bound to organic matter in soil [3]. There is a growing global concern because of the numerous health risks to animals and humans following exposure [4]. Oil-contaminated soils are of environmental concern because they are unsuitable for agricultural and recreational uses and are potential sources for surface and ground water contamination. The PAHs components of the oil have very low water solubility and often tightly bound to soil particles. Oil polluted soil could also become unsuitable due to a reduction in the level of available plant nutrients or a rise to a toxic level of elements such as manganese [5]. This heavy metal content of oil-contaminated soil imposes metabolic disorders and growth inhibition on most of the plant species.

The impacts of metals (cadmium, nickel, zinc, mercury and chromium) on litter decomposition, methanogenesis, acidogenesis and biomass generation have all been studied [6], [7], [8]. Benka-Coker and Ekundayo [9] reported on the impact of zinc, lead, copper and manganese on crude oil biodegradation by a Micrococcus sp. and a Pseudomonas sp. Biodegradation measured by microbial growth was reduced most by zinc and least by manganese.

There have been reports on metal stimulation of bacterial biodegradation processes under favourable environmental conditions of pH, temperature and aerobiosis [6]. Sandrin and Maier [10] observed that such stimulatory effects of metals on biodegradation occurred only when consortia, but not single microbial cultures were used for degradation processes. They argued that stimulation was a result of differential toxicity effects of the tested metals.

The major drawback of a bioremediation exercise is the relatively slow rates at which the process occurs. Heavy metals and pH, among other implicated factors, are on the frontline of this limitation [11], [12], [9]. The manipulation of pH (which curiously has not been well studied) has been suggested as a possible approach to reducing heavy metal toxicity to hydrocarbon-degrading microorganisms [10]. The present study was undertaken to isolate fungi from auto-mechanic workshops and to determine the influence of pH on degradation of waste engine oil co-contaminated with metals (Zn and Pb).

**MATERIAL AND METHODS**

**Collection of Soil Samples**

Soil samples were collected randomly from 3 auto-mechanic workshops at Mgbuka-Nkpor (6°9’N 6°50’E), Nigeria (as described in our previous work) [13], [14]. The hydrocarbon (waste engine oil) used in this work was subsequently collected direct from the engine of 911 Lorry (at Mgbuka-Nkpor) in a sterile container. Uncontaminated soil samples labelled for physicochemical analysis were also collected randomly about 100m from contaminated sites. Samples were transported in cold storage container to the laboratory for analysis.

**Soil Physicochemical Analysis**

Replicate samples labelled for physicochemical analysis were dried at ambient temperature (25°C), crushed in a porcelain mortar and sieved through a 2mm sieve. Air-dried <2mm samples were stored in polythene bags for subsequent analysis. The pH was determined using a calibrated pH meter (Jenway pH meter, 3015 model).

The determinations of Nitrate, Phosphate, Total Nitrogen, Total Hydrocarbon Content (THC) was done according to the method by the American Public Health Association (APHA), [15]. Total Organic Carbon was determined according to the method by Jakobsen, [16]. Exchangeable cations (nutritive salts) were determined according to the method by
Radojevic and Bashin, [17], and the heavy metals were determined by atomic absorption spectrophotometer [18] within 24 hour of sampling.

**Isolation and Screening for Waste Engine Oil Utilizing Fungi**

Waste engine oil utilizing fungi were isolated from soil samples on Mineral Salt agar Medium using the vapour phase transfer method [29], [20]. Each distinct colony on oil degrading enumeration plates were purified by repeated sub culturing onto Sabouraud Dextrose Agar (SDA) (Merck, Germany). The isolates were identified and characterised using colonial appearance and microscopic characteristics based on the schemes of Barnett and Hunter [21] and Efuvwevwere [22]. The isolates were screened for waste engine oil utilization capabilities in mineral salt broth medium.

**Determination of Waste Engine Oil Biodegradation Potentials of the selected isolates**

The rate and extent of biodegradation of waste engine oil by four fungal isolates namely *Candida tropicalis*, *Rhodosporidium toruloides*, *Fusarium oxysporum*, and *Aspergillus clavatus* were assessed using the gravimetric method [23]. Mean results were obtained and expressed as percentage weight loss of engine oil.

**Preparation of Stock Solution of Metal salts**

The metal salts employed in this study include: zinc chloride (ZnCl$_2$) salt and lead trioxonitrate (V) (PbNO$_3$)$_2$ salt. A weight of each of these metal salts that gave a corresponding 1g of each of the respective metal was weighed and dissolved in 100 ml of sterile deionized water and agitated for 10 minutes to ensure complete dissolution and made up to 1 litre with sterile deionized water. Working concentrations of 1, 10, 100 and 1000 mgL$^{-1}$ of the metals were prepared by serial dilutions of the stock solution [24] in sterile deionized water.

**Effect of Zn and Pb on Utilization of Waste Engine Oil at varying pH levels**

The impact of varying concentrations (1, 10, 100 and 1000 mgL$^{-1}$) of metals (Zn and Pb) during utilization of waste engine oil at pH 5.5, 7.0 and 8.5 were determined using the modified method of Ekpenyong and Antai [25]. Tubes of Mineral Salt Medium (MSM) containing waste engine oil (WEO) as carbon source and different concentrations of zinc were inoculated with the pure cultures (*Candida tropicalis* and *Aspergillus clavatus*) as well as the consortium culture of the two isolates and incubated in an orbital shaker at 120rpm at ambient temperature of 28ºC for 16 days. Control tubes which included: control 1 (un-inoculated); MSM + WEO, without inoculum and metal, control 2 (inoculated); MSM + WEO + Inoculum without metal, were also set up. The residual hydrocarbon remaining in the tubes after 16 days was determined using the gravimetric method [23]. The impact of different concentrations of zinc on utilization of waste engine oil at different pH levels was determined from the weight loss (%) of waste engine oil during utilization by the isolates. The whole process was also repeated for lead.

**RESULTS**

There were some observed differences in the physicochemical properties of the contaminated and the uncontaminated samples (Table 1). From the result, the pH of the uncontaminated soil was 5.75% higher than that of the contaminated soil, phosphate concentration was found to be 70.66% higher in uncontaminated soil than in contaminated soil, while the potassium concentration was 5.13% higher in uncontaminated soil than the contaminated soil. However, both total Kjeldahl nitrogen and cadmium concentration was found to be 33.33% higher in contaminated soil than that of the uncontaminated soil, while
magnesium concentration of the contaminated soil was 27.83% higher than that of the uncontaminated soil. Calcium concentration was found to be about 2 fold higher in uncontaminated soil than the contaminated soil, while zinc and copper concentration of the contaminated soil was about 8 fold higher than that of the uncontaminated soil. Lead concentration was 4 fold higher in contaminated soil than that of the uncontaminated soil. Moreover, Total Hydrocarbon Content (THC) of the contaminated soil was found to be 180mg/g while no significant amount of hydrocarbon was detected in the uncontaminated soil (Table 1).

The fungal genera identified in this study were Candida tropicalis, Rhodosporidium toruloids, Fusarium oxysporium, Aspergillus clavatus, Saccharomyces cerevisiae, Candida albicans, Microsporum gypseum and Trichophyton mentagrophytes, based on their cultural and microscopic characteristics. Candida tropicalis, Rhodosporidium toruloids, Fusarium oxysporium and Aspergillus clavatus showed the highest turbidity (+++) and viable count, with an optical density of 1.597, 1.486, 1.422, 1.585 and viable count of 4.3x10^5, 4.1x10^5, 3.0x10^5 and 4.8x10^5 cfu/ml, respectively. Based on these, they were selected for further studies.

The hydrocarbonoclastic potentials of the selected isolates revealed that Candida tropicalis caused 86.2% weight loss of waste engine oil in 16 days. This was followed by 85.0% weight loss caused by Aspergillus clavatus while Rhodosporidium toruloides and Fusarium oxysporium caused 79.3% and 80.5% weight losses, respectively.

The result of the impact of different concentrations of zinc on biodegradation of waste engine oil at varying pH levels are presented in figure 1. An increased inhibition in waste engine oil degradation by Candida tropicalis and Aspergillus clavatus was observed with increasing zinc concentrations. For example, for Candida tropicalis, 10.6% to 25.9% inhibition in waste engine oil degradation was observed as the zinc concentration increased from 1.0 mg/l to 1000 mg/l at pH 5.5. At pH 7.0 and 8.5 respectively, an inhibition in waste engine oil degradation ranged from 7.0% to 22.8% and 16.7% to 36.7% in the presence of 1.0 mg/l to 1000 mg/l zinc concentration (Fig. 1a). For Aspergillus clavatus, an inhibition of 4.7% to 26.6% in the waste engine oil degradation was observed when the zinc concentration increased from 1.0 mg/l to 1000 mg/l at pH 5.5. At pH 7.0, an inhibition of 9.8% to 23.5% in the waste engine oil degradation was observed as the zinc concentration increased from 1.0 mg/l to 1000 mg/l (Fig. 1b). The mixed culture of the isolates achieved a negative inhibition of -10.1% in degradation of waste engine oil, in the presence of 1.0 mg/l zinc concentration at pH 5.5, while at pH 7.0, a negative inhibition of -5.0% in waste engine oil degradation was achieved in the presence of 1.0 mg/l zinc concentration. An inhibition of 4.9% to 26.5% and 8.3% to 16.0% in the waste engine oil degradation was observed when the zinc concentration increased from 10 mg/l to 1000 mg/l at pH 5.5 and 7.0 respectively (Fig. 1c).

The result of the impact of different concentrations of lead on waste engine oil degradation at varying pH levels was presented in figure 2. The result shows that, with Candida tropicalis, a range of 8.9% to 37.7% inhibition in degradation of waste engine oil was observed when the lead concentration increased from 1.0 mg/l to 1000 mg/l at pH 5.5. At pH 7.0, an inhibition of 10.5% to 35.7% in waste engine oil degradation was observed when the lead concentration increased from 1.0 mg/l to 1000 mg/l, while a range of 9.8% to 32.0% inhibition in degradation of waste engine oil was observed when the lead
concentration increased from 1.0 mg/l to 1000 mg/l at pH 8.5 (Fig. 2a). However, with *Aspergillus clavatus*, a negative inhibition (-14.2%) in degradation of waste engine oil was observed in the presence of 1.0 mg/l lead concentration at pH 5.5. When the lead concentration increased from 10 mg/l to 1000 mg/l and at pH 5.5, an inhibition of 3.5% to 25.5% in waste engine oil degradation was observed (Fig. 2b). At pH 7.0 and 8.5 respectively, 4.0% to 27.9% and 9.5% to 30.4% inhibition in degradation of waste engine oil was observed when the lead concentration increased from 1.0 mg/l to 1000 mg/l (Fig 2b). Moreover, with the consortium culture, a range of 15.2% to 34.1% inhibition in waste engine oil degradation was observed when the lead concentration increased from 1.0 mg/l to 1000 mg/l at pH 5.5. At pH 7.0 and 8.5 respectively, 9.3% to 39.7% and 17.1% to 35.0% inhibition in degradation of waste engine oil was observed when the lead concentration increased from 1.0 mg/l to 1000 mg/l (Fig. 2c).

**Table 1. Physicochemical properties of the soil sample**

| Parameters                        | Contaminated soil | Uncontaminated soil |
|-----------------------------------|-------------------|---------------------|
| pH                                | 6.85              | 7.54                |
| Nitrate mg/l                      | 8.421             | 7.763               |
| Phosphate mg/l                    | 6.855             | 11.697              |
| Total Nitrogen %                  | 2.688             | 2.016               |
| Total Organic Carbon %            | 0.174             | 0.163               |
| Total Hydrocarbon Content (THC) mg/g | 180               | ND                  |
| Potassium ppm                     | 18.885            | 19.855              |
| Sodium ppm                        | 9.576             | 9.591               |
| Lead ppm                          | 0.960             | 0.240               |
| Magnesium ppm                     | 21.046            | 16.469              |
| Zinc ppm                          | 8.628             | 1.140               |
| Cadmium ppm                       | 1.762             | 1.324               |
| Copper ppm                        | 2.579             | 0.318               |
| Calcium ppm                       | 32.222            | 73.863              |

ND; Not Detected (≤ 0.001 mg/g)

(a)
Fig. 1: Impact of different zinc concentrations on waste engine oil degradation at varying pH levels. (a): Candida tropicalis, (b): Aspergillus clavatus, (c): consortium culture.
Fig. 2: Impact of different concentrations of lead on waste engine oil degradation at varying pH levels. (a); *Candida tropicalis*, (b); *Aspergillus clavatus*, (c); consortium culture.
DISCUSSION

The physical and chemical properties of soil have been shown to have a reflective influence on aeration, nutrient availability, water retention and consequently on microbial activity [26]. Soils contaminated with petroleum products have been shown to have large increases in nitrogen and phosphate content [27]; [28]. This was in agreement with the findings made on nitrogen determination in this study, whereby the nitrogen content was significantly higher in contaminated soil than in uncontaminated soil, but was not in agreement with the findings on phosphate determination whereby the phosphate content of uncontaminated soil was significantly higher than that of the contaminated soil. This may be related to the extent of contamination, nutrient availability, as well as some soil and microbial properties. Lehtomake and Niemela [29] reported a low value of nitrogen, potassium and phosphorus reserve in petroleum hydrocarbon contamination. This is in line with the observations made for potassium and phosphate determination in this study. An increase in nutrient (nitrate, nitrogen, total organic carbon, magnesium) content observed in contaminated soil was probably due to the effect of contamination with hydrocarbons in the soil. Adenipekun [30] reported a higher level of organic carbon in engine oil contaminated soil compared with the uncontaminated soil. Atlas and Bartha [31] reported that the addition of crude oil to an ecosystem will enrich primarily the microorganisms capable of utilizing the hydrocarbons and secondary microorganisms capable of utilizing metabolites produced by the oil-utilizing microorganisms.

The lower pH observed in contaminated soil compared to the uncontaminated soil in this study was similar to the findings of Osuji and Nwoye [32]. The resulting slightly acidic pH in contaminated soil could be due to the fact that hydrocarbons contain many free cations causing them to have properties of a weak acid. A reduction in pH implies increased acidity which is a problem for agricultural soils because many metal cations are more soluble and available in the soil solution at very low pH including Cd, Cu, Hg, Ni, Pb and Zn [32]. The high concentrations of heavy metals (Zn, Pb, Cu and Cd) observed in contaminated soil compared to the uncontaminated soil used in this study may be attributed to the anthropogenic activities going on in the automechanic workshops. Rapid industrial and domestic activities have caused a concomitant increase in the quantities of metals that are being introduced into the environment [33], thus, resulting in co-contamination of soil with organic and heavy metal pollutants. Waste engine oil is also a source of these metals.

A total of 8 fungal isolates were identified in this study. Of these, 4 isolates that showed high promise for hydrocarbon bioremediation potentials in the screen flask were confirmed as Candida tropicalis, Rhodospiridium toruloides, Fusarium oxysporium and Aspergillus clavatus, using 18S rRNA gene sequencing. Some of these organisms have earlier been reported as hydrocarbon bio-degraders [34]; [35]. Akpoveta et al. [36] reported the isolation of Trichoderma sp., Penicillium sp., Rhizopus sp., Fusarium sp., and Aspergillus sp. from crude oil polluted soil.

Among the 4 isolates that showed high promise for hydrocarbon bioremediation potentials, Candida tropicalis and Aspergillus clavatus displayed the fastest onset and highest extent of biodegradation of waste engine oil. Thus they were selected for further studies. The high rate of hydrocarbon degradation by the two fungi could emanate from their massive growth and enzyme production responses during their growth phases. This could be supported by
the reports of Bogan and Lamar [37], which showed that extracellular ligninolytic enzymes of white rot fungi are produced in response to their growth phases. It was observed that increasing concentrations of the metals studied as well as increasing pH, progressively inhibited the biodegradation of waste engine oil by the pure and consortium culture of the isolates (Candida tropicalis and Aspergillus clavatus) (Figs 1-2). However, zinc at low concentration (1.0 mg/l) exhibited negative (-10.1% and -5.0%) inhibition of waste engine oil degradation at pH 5.5 and 7.0 respectively with the consortium culture (Fig. 1c). This implies stimulation of waste engine oil degradation rather than inhibition. Sandrin and Maier [10], observed that such stimulatory effects of metals on biodegradation occurred only when consortia, but not single microbial cultures were used for biodegradation processes. The stimulation of used engine oil biodegradation by low amounts of zinc at acidic and neutral pH levels in consortium cultures of C. tropicalis and A. clavatus would probably be attributable to the trace requirement of this redox-inactive former of tight complexes [38] by the hydrocarbon degrading enzyme systems of the fungi. Although there was a progressive inhibition of the biodegradation of waste engine oil with increasing concentrations of Pb and increasing pH, in pure and consortium culture of the isolates; (Candida tropicalis and Aspergillus clavatus), there was a 14.2% stimulation of the biodegradation of waste engine oil in the presence of 1.0 mg/l Pb at pH 5.5, with Aspergillus clavatus, (Fig. 2b). Stimulation could be attributed to the differential toxicity effects of the tested metals. Lead in the ionic form (Pb$^{2+}$) has been shown to inhibit aerobic biodegradation of crude oil by Pseudomonas sp. and Micrococcus sp. at 2.80 mg/l and 1.41 mg/l, respectively [9]. Similarly, anaerobic degradation of hexachlorobenzene has been shown to be inhibited by Pb$^{2+}$ at a concentration as low as 0.001 mg/l in microcosms containing contaminated sediments [39]. The pure and consortium culture of the isolates (C. tropicalis and A. clavatus) could be exploited in the bioremediation of waste engine oil polluted soil co-contaminated with low levels of Zn, and Pb at appropriate pH.

ACKNOWLEDGEMENTS

We appreciate the collaboration received from the owners of the sites where samples were collected. We also acknowledge the management of National Agency for Food and Drug Administration and Control (NAFDAC) South East Zonal Laboratory, Agulu, Nigeria, for providing the materials and enabling environment for the study.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

1. Okonokhua BO, Ikhajigbe B, Anoliefo GO. The effects of spent engine oil on soil properties and growth of maize (Zea mays L.), J Appl Sci and Environ Mgt. 2007;11(3): 147-152. www.bioline.org.br/ja

2. Whisman ML, Goetzinger JW, Cotton FO. Radiotracer study of turbine aircraft fuel stability. Air Force Aero Propulsion Laboratory. United States Bureau of Mines. United States of America; 1971.

3. Yong RN, Mohamed AMO, Warkentin BP. Principles of contaminant transport in soil. Elsevier. 1992, 322. https://www.elsevier.com

4. Adams GO, Tawari-Fufeyin P, Igelenyah E, Odukoya E. Assessment of heavy metals bioremediation potential of microbial consortia from poultry litter and spent oil
contaminated site, *Int J Environ Bioremed Biodegrad.* 2014; 2(2): 84-92. [www.sciepub.com](http://www.sciepub.com): Doi:10.12691/ijbhb-4-3-1

5. Udo EJ, Fayemi AAA. The effect of oil pollution on germination, growth and nutrient uptake of corn. *J Environ Qual.* 1975; 4: 537-540. [https://dl.sciencesocieties.org/jeq](https://dl.sciencesocieties.org/jeq)

6. Lin CY. Effect of heavy metals on acidogenesis in anaerobic digestion. *Water Res,* 1993; 27: 147-152. [www.sciencedirect.com](http://www.sciencedirect.com)

7. Bardgett RD, Saggar S. Effects of heavy metal contamination on the short-term decomposition of (14C) glucose in a pasture soil. *Soil Biol Biochem.* 1994; 26: 727-733. [www.journals.elsevier.com](http://www.journals.elsevier.com)

8. Knight BP, Mcgrath SP, Chaudri AM. Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, or zinc. *Appl Environ Microbiol.* 1997; 63: 39-43. [http://aem.asm.org/](http://aem.asm.org/)

9. Benka-Coker MO, Ekundayo JA. Effects of heavy metals on growth of species of *Micrococcus* and *Pseudomonas* in a crude oil/mineral salts medium. *Bioresour Technol.* 1998; 66: 241-245. [www.sciencedirect.com/science/journal/09608524](http://www.sciencedirect.com/science/journal/09608524)

10. Sandrin TR, Maier RM. Impact of metals on the Biodegradation of organic pollutants. *Environ Health Perspectives,* 2003; 111: 1093-1101. doi:10.1289/ehp.5840 available via [http://dx.doi.org/](http://dx.doi.org/)

11. Malakul P, Srivasan KR, Wang HY. Metal Toxicity Reduction in Naphthalene Biodegradation by Use of Metal-Chelating Adsorbents, *Appl and Environ Microbiol.* 1998; 64 (11): 4610–4613. [http://aem.asm.org/](http://aem.asm.org/)

12. Sandrin TR, Maier RM. Effect of pH on cadmium toxicity, speciation, and accumulation during naphthalene biodegradation. *Environ Toxicol and Chem.* 2002; 21: 2075–2079. [http://onlinelibrary.wiley.com](http://onlinelibrary.wiley.com)

13. Mbachu AE, Chukwura EI, Mbachu NA. Isolation and characterization of hydrocarbon degrading fungi from used (spent) engine oil polluted soil and their use for polycyclic aromatic hydrocarbons (PAHs) degradation. *Universal J. of Microbiol. Res.* 2016a; 4(1): 31-37. DOI:10.13189/ujmr.2016.040105 [http://www.hrpub.org/](http://www.hrpub.org/)

14. Mbachu AE, Mbachu NA, Chukwura, EI. 2016b. Biodegradation of N-Alkanes by fungi isolated from waste engine oil polluted soil and their extracellular enzyme activities. *Inter. J. Nov. Res in Life Sci.* 2016b; 3(4): 7-17. [www.noveltyjournals.com](http://www.noveltyjournals.com)

15. APHA. Standard methods for examination of water and wastewater, 20th Edn. Washington, D.C. American Public Health Association; 1998.

16. Jakobsen ST. Chemical reaction and air change during the decomposition of organic matter. *Resour Conserv Recycl.* 1992; 6: 529-539. [www.sciencedirect.com](http://www.sciencedirect.com)

17. Radojevic M, Bashkin VN. Practical environmental analysis. *Royal Soc. Chem.* 1999: 465-466. [www.rsc.org/](http://www.rsc.org/)

18. Oze C, Bird DK, Fendorf S. Genesis of hexavalent chromium from natural sources in soil and groundwater. Proceedings of the National Academy of Sciences of the United States of America (PNAS) 2006; 104: 6544-6549.

19. Hamamura N, Olson S, Ward D, Inskeep W. Microbial population dynamics associated with crude-oil biodegradation in diverse soils. *Appl Environ Microbiol.* 2006; 72: 6316-6324. [http://aem.asm.org/](http://aem.asm.org/)
20. Quatrini P, Scaglione G, Pasquale C, Reila S, Puglia AM. Isolation of Gram-positive n-alkane degraders from a hydrocarbon contaminated Mediterranean shoreline. *J Appl Microbiol.* 2008;104: 251–259. [http://onlinelibrary.wiley.com](http://onlinelibrary.wiley.com)

21. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. Macmillan Publishing Company, New York, 1987.

22. Efiuvwevwere BJO. Microbial spoilage agents of tropical and assorted fruits and vegetables; an illustrative reference book. Port Harcourt, Nigeria: Paragraphics, 2000.

23. Odu CTI, Isinguzo SN. Oil spillage and the use of infant organisms for enhanced biodegradation. The petroleum industry and the Nigerian Environment: Proceedings of 1987 International Seminar Nigerian National Petroleum Corporation, 1979.

24. Zhang S, Crow Jr. SA. Toxic effects of Ag (I) and Hg (II) on *Candida albicans* and *C. maltosa*: A flow cytometric evaluation. *Appl Environ Microbiol.* 2001; 67: 4030-4035. [http://ncbi.nlm.nih.gov/pmc/journals](http://ncbi.nlm.nih.gov/pmc/journals)

25. Ekpenyong MG, Antai SP. Influence of pH on cadmium toxicity to *Bacillus* species (02 and 12) during biodegradation of crude. *Int J Biol Chem.* 2007;1: 29-37. [http://scialert.net/fulltext/doi=ijbc.2007.54.61](http://scialert.net/fulltext/doi=ijbc.2007.54.61)

26. Olaniran AO, Pillay D, Pillay B. Biostimulation and bioaugmentation enhances aerobic biodegradation of dichloroethenes. *Chemosphere*, 2006; 63(4): 600–608. [www.sciencedirect.com](http://www.sciencedirect.com)

27. Odu CTI. Microbiology of Soils Contaminated with Petroleum Hydrocarbons, III. Natural Rehabilitation and Reclamation of Soils Affected. Institute of Petroleum Technol. Publication, India, 1972.

28. Amund OO, Omole CA, Esiobu N, Ugoji EO. Effect of waste engine oil on soil physico-chemical and microbiological properties. *J Sci Res Dev* 1993; 1: 65-68. [http://jsrad.org](http://jsrad.org)

29. Lehtomake M, Niemela S. Improving microbial degradation of oil in soil. *Ambio.*, 1975; 4: 126-129. [www.springer.com/journals/13280](http://www.springer.com/journals/13280)

30. Adenipekun CO. Bioremediation of engine-oil polluted soil by *Pleurotus tuber-regium*, a Nigerian white-rot fungus. *Afri J Biotechnol.* 2008; 7 (1): 55-58. [www.ajol.info/index.php/ajb](http://www.ajol.info/index.php/ajb)

31. Atlas RM, Bartha R. Degradation and mineralization of petroleum by two bacteria isolated from wastewater. *Biotechnol Bioeng.* 1972; 14: 297-308. [http://onlinelibrary.wiley.com/journal/](http://onlinelibrary.wiley.com/journal/)

32. Osuji LC, Nwoye I. An appraisal of the impact of petroleum hydrocarbons on soil fertility: the Òwaza experience. *Afr J Agric Res.* 2007; 2: 318-324. [www.academicjournals.org/ajar/](http://www.academicjournals.org/ajar/)

33. Stephen JR, Chang VR, Macnaughton SJ, Kowalchuk GA, Leung KT, Flemming CA. *et al.* Effect of toxic metals on indigenous soil-subgroup proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. *Appl and Environ Microbiol.* 1998; 65(1): 95–101. [http://aem.asm.org/](http://aem.asm.org/)

34. April TM, Foght JM, Currah RS. Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in Northern and Western Canada. *Can J Microbiol.* 2000; 46(1): 38-49. [www.nrcressearchpress.com/journal/cjm](http://www.nrcressearchpress.com/journal/cjm)

35. Oudot J, Duport J, Haloui S, Roquebert MF. Biodegradation potential of hydrocarbon assimilating tropical fungi. *Soil Biol and Biochem.* 1993; 25: 1167-1173. [www.sciencedirect.com](http://www.sciencedirect.com)
36. Akpoveta OV, Egharevba F, Medjor OW, Osaro KI, Enyemike ED. Microbial degradation and its kinetics on crude oil polluted soil. *Res. J. Chem. Sci.*, 2011; 1(16): 8-14. [www.isca.in](http://www.isca.in)

37. Bogan BW, Lamar R. Polycyclic aromatic hydrocarbon degrading of *Phanerochaete chrysosporium* HHB-1625 and its extracellular ligninolytic enzymes. *Appl Environ Microbiol.* 1996; 62(5): 1597-1603. [http://aem.asm.org/](http://aem.asm.org/)

38. Nies DH. Microbial heavy-metal resistance. *Appl Microbiol Biotechnol.* 1999; 51, 730-750. [www.springer.com](http://www.springer.com)

39. Jackson WA, Pardue JH. Assessment of metal inhibition of reductive dechlorination of hexachlorobenzene at a superfund site. *Environ Toxicol and Chem*, 1998: 17(8): 1441–1446. [http://onlinelibrary.wiley.com](http://onlinelibrary.wiley.com)