

## INSIGHT OF VARIOUS ENVIRONMENTAL DERIVED POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) AND CONSEQUENCES ON LIVING ORGANISMS FROM THE SOIL-A REVIEW

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### ABSTRACT

The environmental destiny of polycyclic aromatic hydrocarbons (PAH) is a significant issue, raising interest in bioremediation. However, the physio-chemical characteristics of PAHs and the physical, chemical, and biological properties of soils can drastically influence in the degradation. Moreover, PAHs are toxic and carcinogenic for humans and their rapid degradation is of great importance. In general the biodegradation of nonchlorinated aliphatic and aromatic hydrocarbons is influenced by their bioavailability. Hydrocarbons are very poorly soluble in water. They are easily adsorbed to clay or humus fractions in the soil, and pass very slowly to the aqueous phase, where they are metabolized by microorganisms. Surfactants that increase their solubility and improve their bioavailability can thereby accelerate degradation. Biodegradation of diesel oil was performed using a diesel oil-degrading bacterial consortium, in both laboratory and pilot scale experiments. Microbial degradation is used as a primary method for the elimination of PAHs from the environment. Although many microorganisms are able to utilize these organic compounds as a substrate and energy source for their growth, these compounds, especially high molecular weight PAHs, are considered as recalcitrant molecules due to their low solubility in water. Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. Purpose of this study was the pollutants encompass negative effects on environmental quality and human health so detoxify or remediate the Hydrocarbonated pollutants from our environment. Apart from this study revealed the significant was after the remediate soil was processed to agricultural

purpose for growing many crops. However, their carving on environment is due to the rapid industrial enterprise of agribusiness, expanded chemical industry and the need to generate cheap forms of energy. The presence of different types of automobiles and machinery has resulted in an increase in the use of lubricating oil.

**KEYWORDS:** Hydrocarbon soil, Xenobiotics, Oil spill, Petroleum, Biodegradation, Environment.

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) represent a large and diverse group of organic compounds. They are stable aromatic hydrocarbon molecules with two or more fused benzene and/or pentacyclic rings in linear, angular or cluster formation (Muckian *et al.*, 2007). PAHs are formed due to anthropogenic pyrolytic processes and also due to natural events such as volcanic eruptions and bush fires. PAHs are distributed ubiquitously in the environment (LaFlamme and Hites, 1978). PAHs produced in the environment are transported over long distances in air before they settle in the atmosphere, on soil surfaces, vegetation, sea and inland waters (Van Jaarsveld *et al.*, 1997).

In recent years, various industrial and/or agricultural processes have been releasing pollutant compounds into the environment (Gianfreda *et al.*, 2004). These pollutants have negative effects on environmental quality and human health. However, their carving on environment is due to the rapid industrial enterprise of agribusiness, expanded chemical industry and the need to generate cheap forms of energy (Borazjani *et al.*, 1997, Borazjani *et al.*, 2000 and Vidali, 2001). The removal of polycyclic aromatic hydrocarbons (PAHs) from the environment has attracted considerable attention owing to their widespread environmental distribution, toxicity, and carcinogenicity (Kou *et al.*, 2009). Microbial degradation is used as a primary method for the elimination of PAHs from the environment. Although many microorganisms are able to utilize these organic compounds as a substrate and energy source for their growth, these compounds, especially high molecular weight PAHs, are considered as recalcitrant molecules due to their low solubility in water. Moreover, they are suspected to be carcinogenic and therefore their rapid remediation is required (Urgun - Demirtas *et al.*, 2006). In recent years, photo-catalysis is one of the most attractive methods for PAHs degradation with respect to solar energy utilization (Kou *et al.*, 2009). Due to the utilization of clean solar energy and no use of any additional chemicals, heterogeneous photo-catalytic processes have gained much interest. To date, most of the studies are dominated by Titanium

oxide (TiO<sub>2</sub>) as a catalyst because of its stability and relatively high activity. Multidisciplinary use of soil, water and sediments leads towards cost effective and efficient remediation techniques. Bioremediation is considered to be one of the important tools for the treatment of contaminants from the environment (Kuiper *et al.*, 2004). Furthermore, degradation of PAHs in contaminated soils are highly influenced by various environmental factors such as pH, water availability, oxygen level, salinity, temperature, PAH bioavailability, nutrient requirements of microbes and adaptation of the microorganism's population (Balba *et al.*, 1991).

Among all the environmental abiotic factors, studies have rarely focused on soil pH and degradation. Soil pH, an abiotic factor, plays an important role in degradation of PAHs. Most PAH-contaminated sites are not at the optimal pH for bioremediation. As pollutants are directly linked to the pH of contaminated sites, the transformation of PAHs under acidic or basic conditions cannot be carried out by indigenous 4 microorganisms. The *in-situ* microorganisms at a contaminated site may be not only tolerant of the site conditions, but may also have the potential to metabolize PAHs in sub-optimal conditions. These facts constitute the necessity for studying the soil pH as important factor (Leahy and Colwell, 1990). The efficiency of bioremediation process depends on the environmental conditions and is generally low (Yan *et al.*, 1998). Considering these conditions in this study, effect of soil pH as an abiotic factor was investigated to monitor the environmental conditions suitable for the promotion of bioremediation. Also, compared to other methods of degradation, soil pH adjustment is an easy process by liming and is a cost effective approach.

Removal of pollutants from the environment can be carried out through remediation processes using one or more of the following: microbial degradation, photo catalytic oxidation, chemical oxidation, volatilization and sedimentation (Gao *et al.*, 1998). It has been observed that industrial and agricultural processes release various polluting compounds in the environment (Gainfreda *et al.*, 2005). These polluting compounds are responsible for negative effects on environmental quality and human health. It is therefore important to control the release of pollutants and understand their fate and effects once they enter the soil. Biodegradation is an inexpensive and an effective approach to degrade and remove pollutants from contaminated soils. In biodegradation, organisms are used to break down and thereby detoxify dangerous chemicals in the environment. As the microbial community structure has been suggested to be important in the decomposition of pollutants (Beulke *et al.*, 2005), there

are different methodologies to degrade these pollutants from the environment. The chemical pollutants can be classified into inorganic and organic pollutants as described below:

### **Organic pollutants and Inorganic pollutants**

Inorganic chemical pollutants in the environment are found naturally. However, due to human activities they may be more concentrated and released into environment. The inorganic pollutants of primary concern are heavy metals such as cadmium, copper, mercury, lead, zinc etc and nutrient pollutants such as nitrogen, phosphates, sulphates (Sandrin & Maier, 2003).

In recent years, many organic compounds are used in day to day life. These organic compounds are produced for different uses such as pesticides, plasticizers, lubricants, refrigerant, fuels, solvents and preservatives (Liu, 2010). Some of these organic compounds are biologically harmful even in very small concentrations but some are relatively inert and harmless. Some of these pollutants that enter into soil may inhibit or kill soil organisms, thereby perturbing the balance of the soil community. However, some may also be transported from the soil to air, water or vegetation where they may come into physical contact, could be inhaled or ingested by number of organisms (Beulke *et al.*, 2005). The key types of organic pollutants are: Aliphatic hydrocarbons Alicyclic hydrocarbons Aromatic hydrocarbons.

### **Aliphatic hydrocarbons**

The Aliphatic hydrocarbon group consists of alkanes, alkenes, and alkynes. The alkanes saturated hydrocarbons (i.e., methane) are fairly inert and generally inactive in atmospheric photochemical reactions (Leahy & Colwell, 1990).

**Saturated hydrocarbons:** If all the hydrogen atoms attached to carbon bonds are together in a chain, molecule is said to be saturated. For example:  $\text{H}_3\text{C}-\text{CH}_3$  (ethane) (Krafft & Crooks, 1988).

**Unsaturated hydrocarbons:** If two adjacent carbon atoms each lose a hydrogen atom, a double bond forms between them. Such a molecule is said to be unsaturated (Krafft & Crooks, 1988). For example:  $\text{H}_2\text{C}=\text{CH}_2$  (ethylene, ethene).<sup>12</sup> Aliphatic and aromatic hydrocarbons are differentiated as they provide a useful method for categorizing these compounds. For example, although anaerobic biodegradation of aromatic hydrocarbons has

been reported, it is uncommon and relatively slow compared to aerobic biodegradation (Leahy & Colwell, 1990). Andrews & Novak (2001) studied the pH effect and the ferrous ion effect on carbon tetrachloride degradation by *Methanosarcina thermophila*. Carbon tetrachloride is considered to be simple unsaturated aliphatic hydrocarbon. Their studies suggest that unsaturated aliphatic hydrocarbons are degraded easier and faster as the pH increases. However, saturated aliphatic hydrocarbons degrade slowly with increase in pH (Whyte *et al.*, 1997).

### **Alicyclic hydrocarbons**

Alicyclic hydrocarbons are made up as cyclic saturated carbon chains. Most common alicyclic hydrocarbons occur naturally. For example, alicyclic hydrocarbons are a major component of crude oil, comprising 20 to 67% by volume. Examples of complex, naturally occurring alicyclic hydrocarbons include camphor, which is plant oil; cyclohexyl fatty acids, which are components of microbial lipids. Rios-Hernandez *et al.*, (2003) studied “Biodegradation of an alicyclic hydrocarbon by a sulfate-reducing enrichment from a gas condensate-contaminated aquifer.” Ethylcyclopentane (ECP) which is an alicyclic hydrocarbon was used in the research conducted to study its metabolism by sulfate-reducing bacterial enrichment. Moreover, the research suggests that (ECP)-alicyclic hydrocarbons are anaerobically activated by addition of fumarate. Alkylsuccinate derivatives are obtained as by-products under sulfate-reducing conditions.

### **Aromatic hydrocarbons**

Aromatic hydrocarbons are found in petroleum components and its refined products. Naturally occurring aromatic hydrocarbons consists of benzene and substituted derivatives of benzene. Benzene is an aromatic compound exhibiting similar chemical behaviour and is one of the simplest forms of petrochemicals (Krafft & Crooks, 1988). Naphthalene is considered as one of the simplest representative of polycyclic aromatic hydrocarbons and benzene, toluene and ethyl benzene are among the other important aromatic petroleum hydrocarbons (Wrenn *et al.*, 1998). Benzene exhibits important properties and is a naturally occurring of aromatic hydrocarbons. The elemental composition of benzene is organic compound with molecular composition of C<sub>6</sub>H<sub>6</sub> with six-member ring and with three carbon-carbon double bonds. Also, due to delocalised nature of bonding, benzene is represented with ring inside hexagonal arrangements of carbon atoms (Wilson and Jones, 1993). It is structurally similar to cyclic alkenes and is cyclic in nature. It is colourless and highly flammable. Benzene is

considered as an aromatic hydrocarbon and is naturally occurring constituent of crude oil. It is, however, unusually stable and does not readily participate in reactions that are characteristic of alkenes (Wrenn *et al.*, 1998). Many chemical compounds are originated from benzene by substituting one or more of its hydrogen atoms with some other functional group. The resulting effect on the reactivity of these molecules that distinguishes aromatic hydrocarbons from unsaturated aliphatic hydrocarbons depends on the relative stability of benzene and its derivatives (Rios-Hernandez *et al.*, 2003).

**PAHs:** Polycyclic Aromatic Hydrocarbons (PAHs) are a class of stable organic molecules which consist of hydrogen and carbon molecules. As PAHs are commonly found widespread contaminants, these are of environmental concern (Uyttebroek *et al.*, 2007). The structure of PAHs compounds appears flat and consists of carbon and hydrogen atoms (Cutright, 2006). However, other atoms like sulphur, nitrogen and oxygen get readily substituted in the benzene ring and get converted to heterocyclic aromatic compounds. These heterocyclic aromatic compounds are commonly grouped together with PAHs (Wilson & Jones, 1993). Also, unsubstituted PAHs are non-polar, neutral and hydrophobic compounds that are randomly scattered during energy conversion and industries dealing with petroleum (Juhasz *et al.*, 2000).

#### **Biodegradation of hydrocarbons in soil by microbial syndication**

Biodegradation of complex hydrocarbon usually requires the cooperation of more than a single species. This is particularly true in pollutants that are made up of many deferent compounds such as crude oil or petroleum and complete mineralization to CO<sub>2</sub> and H<sub>2</sub>O is desired. Individual microorganisms can metabolize only limited range of hydrocarbon substrates, so assemblages of mixed populations with overall broad enzymatic capacities are required to bring the rate and extent of petroleum biodegradation further. Microbial populations that consist of strains that belong to various genera have been detected in petroleum-contaminated soil or water (Sorkhoh *et al.*, 1995). This strongly suggests that each strain or genera have their roles in the hydrocarbon transformation processes.

Venkateswaran and Harayama (1995) reported similar observations in sequential enrichments in medium containing residual crude oil. In an earlier study using pure cultures, it was reported that after exhaustive growth of one strain on crude oil, the residual oil supported the growth of a second and third strain of bacteria (Horowitz *et al.*, 1975). Following the above findings, many studies of petroleum transformation have employed mixed bacterial or

bacterial– fungal cultures in efforts to maximize biodegradation. Rambeloarisoa *et al.* (1984) demonstrated a consortium of 8 strains made up of members of 6 genera to be able to electively degrading crude oil. Interestingly, only 5 of these strains were able to grow in pure cultures using a variety of hydrocarbons. However, when the other 3 strains were removed from the consortium, the effectiveness of the mixed culture was remarkably reduced. This further supports the theory that each member in a microbial community has significant roles and may need to depend on the presence of other species or strains to be able to survive when the source of energy is limited and confined to complex carbons the capacities of the individual strains forming the association.

### **Efficacy of soil degradation by Microbial Organisms.**

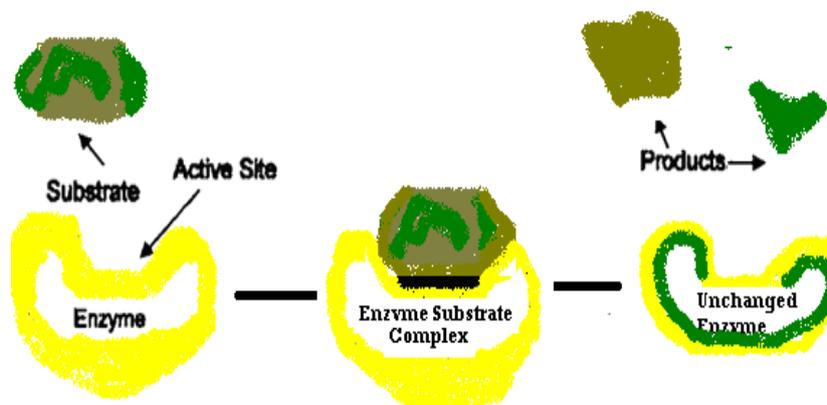
Several petroleum aliphatic and polycyclic aromatic hydrocarbons can act as source of carbon and energy for the growth of soil microorganisms Bouwer 1997. One main factor that influences the extent of their Biodegradation is their bioavailability and this is a priority research objective in the bioremediation field (Horowitz *et al.*, 1975). Their hydrophobic and low water solubility mean that hydrocarbons pass very slowly from a non-aqueous to the aqueous phase liquid in which they are metabolised by microorganisms (Moody *et al.*, 2001). Moreover, in the soil they are adsorbed to clay or humus fractions (Muckian *et al.*, 2007).

### **Enzymes produced by soil microorganisms**

Soil enzymes are proteins which catalyze different chemical reactions by increasing the rate of reaction. In a soil enzyme reaction, molecules at an initial level considered as substrates, are converted molecules are termed as products as shown in figure 1. Different types of chemical reactions in the biological systems of cell require enzymes to gain considerable rate.

The degradative capacity of any microbial consortium is not necessarily the result of merely adding together of Many groups researching consortia biodegradation observed this. Again Komukai–Nakamura and co-workers (1996) reported the sequential degradation of Arabian light crude oil by two different genera. *Acinetobacter* sp. T4 was biodegraded alkanes and other hydrocarbons producing the accumulation of metabolites. Following that, *Pseudomonas putida* PB4 began to grow on the metabolites and finally degrade aromatic compounds in the crude oil (Yakimov *et al.*, 1999). The advantages of employing mixed cultures as opposed to pure cultures in bioremediation have also been widely demonstrated. It is possible that one species removes the toxic metabolites (that otherwise may hinder microbial activities) of the species preceding it. It is also possible that the second species are able to degrade compounds

that the First are able to only partially (Alexander, 1999). Further research should be directed towards understanding the roles of individual members in influencing the effectiveness of a microbial association.



**Figure 1: Process of Enzymatic reaction by Microbial degradation in soil**

### **Monitoring and Characterization of Aromatic Hydrocarbon Degrading Bioaugmented microorganisms from Petroleum Contaminated Soil**

Hydrocarbons are common environmental pollutants with toxic, genotoxic, mutagenic and carcinogenic properties (Mastrangela *et al.*, 1997). They mainly occur in petroleum industry activities (Blumer, 1976). Oil spills because of pipeline breakages, tanks leakages or storage and transportation accidents can be considered as the most frequent causes of hydro-carbon release, included PAHs into soils (Bossert *et al.*, 1984). BTEX compounds are components of gasoline and aviation fuels that are carcinogenic and neurotoxic to most organ-isms (Levin and Forchiassin, 2003). Bacteria play a major role in hydrocarbon degradation. The reason for petroleum biodegradation is the ability of microorganisms to utilize hydrocarbons to satisfy their cell growth and energy needs. Low molecular weight alkanes are degraded most rapidly whereas mixed cultures carry out more extensive biodegradation of petroleum through pure cultures (Ghazali *et al.*, 2004; Trindada *et al.*, 2004). Therefore a biodegradation using microorganism is usually preferred. They play major role in PAHs removal from contaminated environments because of some advantages such as cost effectiveness and more complete cleanup (Pothuluri and Cerniglia, 1994).

Previously, Nnamchi *et al.*, 2006 depicted twenty four bacteria capable of utilizing naphthalene as their sole source of carbon and energy for growth from three different soils in

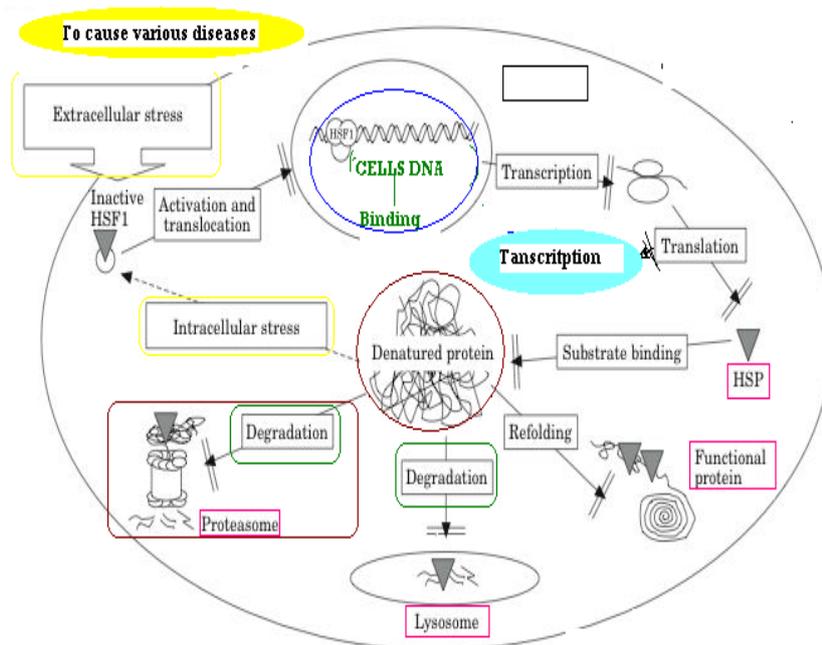
Nsukula, Nigeria were isolated and characterized. The usage of petroleum hydro-carbon products has been increased. Therefore, the soil contamination with diesel and engine oil is becoming one of the major environmental problems. Various microbial species are effective degraders of hydrocarbons in natural environment. Microorganisms such as bacteria, fungi, yeast and microalgae can degrade petroleum hydrocarbons (Roberts, 1992; Bundy *et al.*, 2004).

Today, we all acknowledge the significance of pollutants in the air or in water contributing to poor health. Measures of air quality are often reported along with our daily weather, and the impacts of a lack of access to safe drinking water, or of industry discharging pollution into rivers and lakes, are well documented. In many cases, clear links have been drawn between the types and levels of specific contaminants in the air or water, and their health effects. However, until recently, the impacts of soil pollution on our health have had a much lower profile. In addition, the science involved is complex (Science for Environment Policy, 2012). Researchers are making good progress with developing our understanding of many soil-related issues, such as soil sealing, erosion and contamination, but the impacts of soil contamination on our health are not as well documented (De Jonge *et al.*, 1997). This report aims to begin filling this gap in information for decision makers, with a particular focus on offering explanations of the scientific issues around how soils behave, details of common contaminants in our soils, and what we know about the potential risks to health from soil contamination.

### **Biological Impacts of Organic pollutants in soil**

In a European context, one topic of particular concern is citizen's long term, low-level exposure to a range of soil contaminants, including both current and legacy (historical) emissions. Cases of populations suffering from high levels of soil contamination in specific locations around the world have been studied extensively by epidemiologists and toxicologists to understand the health impacts of soil-borne chemicals in the environment (Galli, 1998). In these cases, the cause and effect are often relatively straightforward to determine. However, the effects of living for many years on or near soils with above-average levels of contamination can be harder to determine by Bai *et al.*, (1997). The study of soils and human health is a complicated endeavor: traditional scientific approaches that isolate a single variable, such as a specific contaminant, and then investigate that variable are not effective in this case, because many of the issues that affect human health involve

complicated and synergistic relationships (Brevik *et al.*, 2013). This report focuses primarily on soil contaminants from human activity, for example, from industrial processes, mining, and household/business waste, human and animal pharmaceuticals. It provides an overview of current research and presents case studies concerning heavy metals and synthetic organic chemicals. Soil also contains a great number of biological contaminants (e.g. pathogens, such as tetanus, and parasites, such as hookworm), which cause many well-documented impacts on human health (Fig.2).



**Figure: 2- Biological Effect of Hydrocarbonated Waste from Oil Spilling Area**

## CONCLUSION

Environmental pollution was increased in every year by petroleum and petroleum related compounds mainly diesel oil. Soil and water are often contaminated with diesel oil leakage from pipelines, storage tanks, accidental oil spills, etc. The world production of crude oil was increasing more than 3 billions /year. Diesel oil is highly complex in nature; it is very difficult to understand the degradation mechanism. Diverse group of microorganisms have the ability to clean up the hydrocarbon contaminated sites. Soils will lead to soils with different physical and chemical properties. A soil's unique composition will affect how much water it can hold, the living organisms it supports, which chemical reactions are likely to occur, and how it cycles nutrients. The ability of various indigenous bacteria, especially those isolated from contaminated sites, to metabolize crude oil or aliphatic hydrocarbons is well known. Several experiments were demonstrated that crude oil degrading microorganisms are not restricted to oil polluted sites. This finding supports the fact that crude oil degrading

microorganisms are widely distributed in the environment, and therefore can be “easily” collectable from sites with no apparent history of crude oil pollution as it was in our case. All of these factors will determine what happens to potentially harmful contaminants in soils, how they may be transported or transformed, and the extent to which they may be available in chemical forms that are harmful to human health. But due to the highly toxic potential of this class of compounds, reducing current background exposure is advisable.

## REFERENCES

1. Alexander, M. 1999. Biodegradation and Bioremediation, 2nd Edition. Academic Press, San Diego.
2. Bai G, Brusseau ML, Miller RM. Biosurfactant enhanced removal of residual hydrocarbon from soil. *Journal of Contaminant Hydrology* 1997; 25: 157–70.
3. Balba, A. M., Elshibiny, G. and Elkhatib, E. S. (1991). Effect of Lead Increments on the Yield and Lead Content of Tomato Plants. *Water Air and Soil Pollution*, 1991; 57-8: 93-99.
4. Bamforth, S. M. and Singleton, I. (2005). Naphthalene transformation by the *Pseudomonas* at an elevated pH. *Journal of Chemical Technology and Biotechnology*, 2005; 80(7).
5. Beulke, S., Van Beinum, W., Brown, C. D., Mitchell, M. and Walker, A. 2005. Evaluation of simplifying assumptions on pesticide degradation in soil. *Journal of Environmental Quality*, 2005; 34(6): 1933-1943.
6. Blumer, M. 1976 “Polycyclic Aromatic Hydrocarbons in Nature,” *Scientific American*, 234 (1): 34-44.
7. Brevik, E.C. & Burgess, L.C. eds 2013. *Soils and Human Health*. Boca Raton: CRC Press.
8. Borazjani, R. N., May, L. L., Noble, J. A., Avery, S. V. and Ahearn, D. G. (2000). Flow cytometry for determination of the efficacy of contact lens disinfecting solutions against *Acanthamoeba* spp. *Applied and Environmental Microbiology*, 2000; 66(3): 1057-1061.
9. Bossert, M. Kachel W. and Bartha, R. 1984, “Fate of Hydro-carbons during Oily Sludge Disposal in Soil,” *Applied Environment Microbiology*, 1984; 47(4): 763-767.
10. Bundy, J. G. Paton G. I. and Campbell, C. D. 2004. “Combined microbial Community Level and Single Species Biosensor Responses to Monitor Recovery of Oil Polluted Soil,” *Soil*
11. Cutright, T. J. and Hwang, S. 2006. Polycyclic Aromatic Hydrocarbons (PAHs). *Encyclopedia of Chemical Processing*, 2006; 2291-2299.

12. De Jonge H, Freijer JI, Verstraten JM, Westerveld J, Van der Wielen FWM. Relation between bioavailability and fuel oil hydrocarbon composition in contaminated soils. *Environ Sci Technol* 1997; 31 :771–5.
13. Farinazleen M. G., Raja N. Z. Abdul R., Abu B. S., Mahiran B., 2004. Biodegradation of hydrocarbons in soil by microbial consortium, *International Biodeterioration and Biodegradation*, 2004; 54: 61 – 67.
14. Mastrangelo, G. Fadda E. and Marzia, 1997. “Polycyclic Aromatic Hydrocarbons and Cancer in Man,” *Environment Health Perspect*, 1997; 104(11): 1166- 1170.
15. Gao, J. P., Maguhn, J., Spitzauer, P. and Kettrup, A. (1998). Sorption of pesticides in the sediment of the Teufelsweiher Pond (Southern Germany). I: Equilibrium assessments, effect of organic carbon content and pH. *Water Research*, 1998; 32(5): 1662-1672.
16. Galli E. 1998. Evolution of new degradative pathways in microorganisms of environmental interest. In: *Biotechnology for soil remediation. Scientific bases and practical applications*. R. Serra. C.I.P.A. S.r.l., Milan, Italy, 67–80.
17. Ghazali, M. F. Zakha, N. R. Abdul, R. N Salleh A. B. and Basri, M. 2004. “Biodegradation of Hydrocarbons in Soil by Microbial Consortium,” *International Biodeterioration and Biodegradation*, 2004; 54(1): 61-67.
18. Gianfreda, L., Luz Mora, M. and Cristina, M. 2005. Restoration of polluted soils by means of microbial and enzymatic processes. Review article: 20-40.
19. Gianfreda, L., Luz Mora, M. and Cristina, M. 2005. Restoration of polluted soils by means of microbial and enzymatic processes. Review article: 20-40.
20. Horowitz, A., Gutnick, D., Rosenberg, E., 1975. Sequential growth of bacteria on crude oil. *Applied Microbiology*, 1975; 30: 10–19.
21. Juhasz, A. L. and Naidu, R. 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. *International Biodeterioration & Biodegradation*, 2000; 45(1-2): 57-88.
22. Komukai–Nakamura, S., Sugiura, K., Yamauchi-inomata, Y., Toki, H., Venkateswaran, K., Yamamoto, S., Tanaka, H., Harayama, S. 1996. Construction of bacterial consortia that degrade Arabian light crude oil. *Journal of Fermentation and Bioengineering*, 1996; 82: 570–574.
23. Kou, J., Li, Z., Yuan, Y., Zhang, H., Wang, Y. and Zou, Z. 2009. Visible-light-Induced photocatalytic oxidation of polycyclic aromatic hydrocarbons over tantalum oxynitride photocatalysts. *Environmental Science Technology*, 2009; 43(8): 2919-2924.

24. Krafft, M. E. and Crooks. III W. J.1988. Saturated and unsaturated hydrocarbons; Review article. *Journal of Organic Chemistry*, 1988; 53: 3158-3163.
25. Kuiper, I., Lagendijk, E. L., Bloemberg, G. V. and Lugtenberg, B. J. 2004. Rhizoremediation: a beneficial plant-microbe interaction. *Molecular Plant Microbe Interaction*, 2004; 17(1): 6-15.
26. LaFlamme, R. E. and Hites, R. A. 1978. The global distribution of polycyclic aromatic hydrocarbons in recent sediments. *Geochimica et Cosmochimica Acta*, 1978; 42: 289-303.
27. Leahy, J. G. . Tracy, K. D Eley M. H. 2003 “Degradation of Mixtures of Aromatic and Aliphatic Hydrocarbons by Aromatic Hydrocarbon-Degrading Bacteria,” *FEMS-Microbiology-Ecology*, 2003; 43(2): 271-276.
28. Leahy, J. G. and Colwell, R. R. 1990. Microbial-Degradation of Hydrocarbons in the Environment. *Microbiological Reviews*, 1990; 54(3): 305-315.
29. Levin L. A. and Forchiassin, V. A. 2003. “Degradation of Or-ganic Pollutants by the White Rot Basidiomycete *Tram-etes Trogii*,” *International Biodeterioration & Biodegradation*, 2003; 52: 1-5.
30. Liu, W. W., Yin, R., Lin, X. G., Zhang, J., Chen, X. M., Li, X. Z. and Yang, T. 2010. Interaction of biosurfactant-microorganism to enhance phytoremediation of aged polycyclic aromatic hydrocarbons (PAHS) contaminated soils with alfalfa (*Medicago sativa* L.). *Huan Jing Ke Xue*, 2010; 31(4): 1079-1084.
31. Moody, J. D., Freeman, J. P., Doerge, D. R. and Cerniglia, C. E. 2001. Degradation of phenanthrene and anthracene by cell suspensions of *Mycobacterium* sp. strain PYR-1. *Applied Environmental Microbiology*, 67(4): 1476-1483.
32. Muckian, L., Grant, R., Doyle, E. and Clipson, N. 2007. Bacterial community structure in soils contaminated by polycyclic aromatic hydrocarbons. *Chemosphere*, 2007; 68(8): 1535-1541.
33. Nnamchi, I., Obeta A. N and Ezeogu, L. I. 2006, “Isolation and Characterization of Polycyclic Aromatic Hydrocarbon Degrading Bacteria from Nsukka Soils in Nigeria,” *International Journal of Environmental Science and Tech-nology*, 2006; 3(2): 181-190.
34. Pothuluri J. V. and Cerniglia C. E. 1994. “Microbial Metabolism of Polycyclic Aromatic Hydrocarbons,” In: G. R. Chaudry, Ed., *Biological Degradation and Bioremedia-tion Toxic Chemicals*, Chapman and Hall, London: 1994, pp. 92-124.

35. Rambeloarisoa, E., Rontani, J.F., Giusti, G., Duvnjak, Z., Bertrand, J.C., 1984. Degradation of crude oil by a mixed population of bacteria isolated from sea-surface foams. *Marine Biology*, 1984; 83: 69–81.
36. Rios-Hernandez, L. A., Gieg, L. M. and Suflita, J. M. 2003. Biodegradation of an alicyclic hydrocarbon by a sulfate-reducing enrichment from a gas condensate-contaminated aquifer. *Applied Environmental Microbiology*, 2003; 69(1): 434-443.
37. Roberts, E. R. 1992. “Bioremediation of Petroleum Contaminated Sites,” RC Press Inc., Boca Raton.
38. Sandrin, T. R. and Maier, R. M. 2003. Impact of metals on the biodegradation of organic pollutants. *Environmental Health Perspectives*, 2003; 111(8): 1093-1101.
39. Science for Environment Policy 2012. European Commission DG Environment News Alert Service, edited by SCU, The University of the West of England, Bristol. DG ENV News Alert Issue 288, 15 June 2012. Raising the profile of soil’s essential contribution to society.
40. Shakoori R. and Zahra, B. 1999. “Hydrocarbon Degrading Bacteria Isolated from Soil Samples and Industrial Effluents and Their Potential Use in Environmental Cleanup,” *Proceedings of Pakistan Congress of Zoology*, 1999; 19: 309-338.
41. Sorkhoh, N.A., Al-Hasan, R.H., Khanafer, M., Radwan, S.S. 1995. Establishment of oil-degrading bacteria associated with cyanobacteria in oil-polluted soil. *Journal of Applied Bacteriology*, 1995; 78: 194–199.
42. Trindada, P. V. O. Sobral, L. G. Rizzo, A. C. L. Leitic S. G. F. and Soriano, A. U. 2004 “Bioremediation of a Weathered and Recently Oil Contaminated Soils from Brazil—A Comparison Study,” *Chemosphere*, Vol. 58, No. 4, 2004, pp. 515-522. doi:10.1016/j.chemosphere.09.021.
43. Urgan-Demirtas, M., Stark, B. and Pagilla, K. 2006. Use of genetically engineered microorganisms (GEMs) for the bioremediation of contaminants. *Critical Reviews in Biotechnology*, 2006; 26(3): 145-164.
44. Uyttebroek, M., Vermeir, S., Wattiau, P., Ryngaert, A. and Springael, D. 2007. Characterization of cultures enriched from acidic polycyclic aromatic hydrocarbon-contaminated soil for growth on pyrene at low pH. *Applied and Environmental Microbiology*, 2007; 73(10): 3159-3164.
45. VanJaarsveld, A. J., Vanpul, W. J. and De Leeuw, F. A. 1997. Modelling transport and deposition of persistent organic pollutants in the European region. *Atmospheric Environment*, 1997; 31: 1011-1997.

46. Venkateswaran, K., Harayama, S., 1995. Sequential enrichment of microbial populations exhibiting enhanced biodegradation of crude oil. *Canadian Journal of Microbiology*, 1995; 41, 767–775.
47. Vidali, N. 2001. Bioremediation. A review. *Pure Applied Chemistry* 73: 1163-1172.
48. Whyte, L. G., Bourbonniere, L. and Greer, C. W. 1997. Biodegradation of petroleum hydrocarbons by psychrotrophic *Pseudomonas* strains possessing both alkane (alk) and naphthalene (nah) catabolic pathways. *Applied Environmental Microbiology* 63(9): 3719-3723.
49. Wilson, S. C. and Jones, K. C. 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. *Environmental Pollution*, 1993; 81(3): 229-249.
50. Wrenn, B. A., Suidan, M. T., Strohneier, Eberhart, L. and G. J. Wilson. (1998). Influence of tide and waves on washout of dissolved nutrients from the bioremediation zone of a coarse-sand beach: Application in oil-spill bioremediation. *Spill Science & Technology*, 1998; (4): 99-106.
51. Yan, S. C., Ouyang, S. X., Gao, J., Yang, M., Feng, J. Y., Fan, X. X., Wan, L. J., Li, Z., S., Ye, J. H., Zhou, Y. and Zou, Z. G. 1998. A room-temperature reactive-template route to mesoporous ZnGa<sub>2</sub>O<sub>4</sub> with improved photocatalytic activity in reduction of CO<sub>2</sub>. *Angewandte Chemie International Edition*, 1998; 49(36): 6400-6404.
52. Yakimov MM, Giuliano L, Bruni V, Scarfi S, Golyshin P.N. Characterization Of Antarctic Hydrocarbon-Degrading Bacteria Capable Of Producing Bioemulsifier. *New Microbiol.* 1999; 22 (3):249 –56.