

## Bioremediation of hydrocarbon-polluted soils for improved crop performance

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### ABSTRACT

Soil pollution arising from increasing demand for petroleum and its products has become a common problem in recent years. Soils polluted with petroleum hydrocarbons (PHCs) differ from unpolluted soils and are not able to support adequate crop growth and development. There is need to treat these soils so as to satisfy the food requirement of the ever increasing world population. Bioremediation is a cost-effective method of soil remediation which uses organisms for the treatment of polluted soils. It has been used across the globe for the treatment of a wide range of organic soil pollutants. In this paper the different approaches to bioremediation of PHC-polluted soil have been discussed. It is evident that the combined use of both microorganisms and phytoremediators improves the efficiency of bioremediation. More research on the interactions between the remediating organisms and the environment is needed for better understanding of this approach to bioremediation.

**Keywords:** bioremediation, petroleum hydrocarbons, soil properties, crop production, phytoremediation, mycorrhizae.

### 1. Introduction

Hydrocarbon-polluted soils are widespread across the globe due to increase in the demand for petroleum as an energy source. Petroleum (crude oil) and its product enter the soil via crude oil pipe leakages, oil tank ruptures and indiscriminate disposal of refinery products (Schwab and Banks, 1999) leading to changes in soil properties (McGill *et al.*, 1981; Trofimov and Rozanova, 2003). Soils polluted with petroleum hydrocarbon (PHC) are low in fertility and hence, do not support adequate crop growth and development (Abii and Nwosu, 2009).

Various methods exist for the treatment of PHC-polluted soils. They range from the conventional methods of excavating and removal of the affected soils to landfills, capping of the affected areas in a site, stabilizing the soil with materials such as lime, apatite and cement, incineration to chemical methods involving the use of compounds such as potassium permanganate, sodium per sulphate and hydrogen peroxide, washing of soil with strong acids and more recently, to the use of organisms (bioremediation).

The conventional methods which may appear to require less cost and expertise do not solve the problem entirely. For instance, the method of excavating and dumping of polluted soil in landfills has become uncommon because of the risks associated with excavating and transporting the polluted soil. Landfills are also difficult to find nowadays and when seen, are rather expensive. Furthermore, this method does not solve the problem when viewed from a

larger perspective; it merely shifts it to a different location. Capping the polluted areas of a site requires close monitoring of the soil for as long as it remains polluted and this may not be so easy. Incineration, on the other hand, is not an environmentally friendly approach to soil remediation since it discharges high amounts of CO<sub>2</sub> and thus contributes to climate change. The use of chemicals is a very fast method of soil remediation. However, the costs needed for the implementation of this method are usually too high and the chemicals may react with other beneficial soil components.

Bioremediation is a widely accepted method of remediation because it is an environmentally friendly method that requires less cost and techniques. To a large extent, bioremediation equally accomplishes complete clean-up of the polluted soil. When compared with other conventional remediation techniques, results have shown that bioremediation reduces PHC concentrations in polluted soils with minimum site disturbance and at lower costs (Buzea and DeStefanis, 2001). This paper focuses on the various approaches to bioremediation for the treatment of PHC-polluted soil with a view of improving the soils for better crop production.

## 2. What are hydrocarbons?

Hydrocarbons are organic compounds which contain only carbon and hydrogen. Structurally, they can be grouped into two broad classes: the aliphatic hydrocarbons and the aromatic hydrocarbons. Aliphatic hydrocarbons are best known as straight chained hydrocarbons. They are further grouped into the saturated hydrocarbons (alkanes) and the unsaturated hydrocarbons (alkenes and alkynes). Aliphatic hydrocarbons can also be cyclic in nature (cycloalkanes). Aromatic hydrocarbons, also known as arenes, contain at least one aromatic ring. A typical example of an aromatic hydrocarbon is benzene. Aromatic hydrocarbons are more water soluble than aliphatic hydrocarbons of the same equivalent carbon number (EC). Aliphatic hydrocarbons on the other hand, are more volatile and more attached to organic matter than aromatic hydrocarbon (Gustafson *et al.*, 1997; Palmroth, 2006).

Hydrocarbons are the primary components of crude oil. Their composition varies from 50 – 98% depending on the type of crude oil and the method of extraction. Other organic compounds found in crude oil include nitrogen, oxygen and sulphur. These compounds make up 6 – 10% of crude oil. Inorganic elements and heavy metals such as copper, iron, boron, molybdenum, cadmium, lead, vanadium and nickel occupy less than 1% of total composition of crude oil (FGS, 2001). Hydrocarbons are thus the major components of crude oil. This is because decomposed organic matter – the parent material of crude oil – contains an abundance of carbon and hydrogen. Hydrocarbons can be solids/waxes (e.g. naphthalene), liquid (e.g. benzene), gases (e.g. methane), or polymers (e.g. polyethene).

### 2.1. Behaviour of hydrocarbons in soils

Hydrocarbons that escape into the environment act differently depending on two main factors, namely: their chemical composition and the environment they enter (Taylor, 2007). According to McGill *et al.* (1981), the extent of physical movement of PHC in the soil profile depends on viscosity of the hydrocarbon, soil temperature, soil moisture content, soil structure and texture. Hydrophobicity, insolubility and extent of sorption to soil increase with increase in size and complexity of hydrocarbons (Cerniglia and Sutherland, 2001; Chaineau *et al.*, 2003). PHCs tend to move horizontally within the soil under cold conditions. During high temperature and dry soil condition on the other hand, vertical movement into the water saturated areas supersedes lateral movement (McGill *et al.*, 1981).

PHCs that enter the soil may be adsorbed on the surface of mineral and organic soil constituents, fixed within the soil pores and fissures, found in mobile form or may form a continuous cover on the soil surface (Trofimov and Rozanova, 2003). Chemical retention of PHC in soils depends on type and concentration of solute in surrounding solution, type of clay minerals, cation exchange capacity, soil organic matter content, soil pH and temperature (Alexander, 1999; Semple *et al.*, 2003). Suleimanov *et al.* (2005) found that in forest soils, heavier fractions of PHC were retained in the eluvial horizons that contained the larger pores, while, the lighter fractions were retained in the illuvial horizon that contained the fine water retention pores.

## 2.2. Effect of hydrocarbons on soil properties

Soils that are polluted with PHCs are different from unpolluted soils due to changes in their biological as well as physicochemical properties (Robertson *et al.*, 2007). When a soil is polluted with PHC, an initial reduction in soil microorganisms is observed (especially in soils that have not been previously polluted). However, this reduction is followed by a rapid increase in the number of microorganisms that are capable of degrading the contaminants (Gramss *et al.*, 1998; Seghers *et al.*, 2003). Hofman *et al.* (2004) recorded that though the number of soil microorganisms increase in PHC-polluted soils, species richness often decreases over time. PHC may interfere with plant-fungus relationship by altering the soil environment so that movement of diffusible chemical signals such as auxins is prevented. It may also affect this relationship by altering the root exudation pattern (Kirk *et al.*, 2005). Burrowing soil organisms may be affected by the reduced or total absence of aeration in hydrocarbon-polluted soils. However, in soils with high humus content, toxicity of PHC on soil organisms have been found to be less severe (Salminen and Haimi, 1997). Nicolotti and Egli (1998) recorded that some fungi may appear resistant to PHC. They added that these organisms may even benefit from the presence of the pollutants.

The hydrophobic nature of PHCs influences the water holding capacity (WHC) and moisture content of soils. Studies have shown that soils polluted with PHCs are characterized by lower WHC, moisture content and hydraulic conductivity compared with unpolluted soils (Trofimov and Rozanova, 2003; Suleimanov *et al.*, 2005; Nwaoguikpe, 2011) (Table 1). Increased soil structural stability due to PHC contamination has also been documented (McGill *et al.*, 1981; Certini, 2005). Akpoveta *et al.* (2011) observed that there was no effect on soil texture after PHC contamination.

Reduced soil pH together with increases in soil organic carbon and organic matter on crude oil polluted soils have been recorded (Osuji and Nwoye, 2007; Marinescu *et al.*, 2011; Nwaoguikpe, 2011) (Table 1). Increases in soil sodium and iron have also been observed on these soils (Obire and Nwaubete, 2002; Trofimov and Rozanova, 2003). Pollution with PHC does not always have a specific effect on soil properties. For instance, while Marinescu *et al.* (2011) observed an increase in total nitrogen on a crude oil polluted soil, Obire and Nwaubete, (2002) observed a reduction in soil nitrogen on a similar soil. Similarly, while Akpoveta *et al.* (2011) recorded a reduction in soil phosphorus on a PHC-polluted soil, Marinescu *et al.* (2011) recorded an increase in the same element on a similar soil. These discrepancies may be attributed to the nature of the polluting substance as well as the initial soil properties (McGill *et al.*, 1981; Alexander, 1999; Semple *et al.*, 2003).

**Table 1:** Comparison of crude oil polluted and unpolluted soils

Location 1: Owerri, Imo State, Nigeria (Nwaoguikpe, 2011)		
Parameter	Polluted	Unpolluted
Moisture content (%)	4.23	7.99
Bulk density (gcm <sup>-3</sup> )	1.28	1.36
	4.40	5.30
K (mgkg <sup>-1</sup> )	105.78	35.45
Na (mgkg <sup>-1</sup> )	193.84	76.46
Organic matter (%)	3.35	2.10
Location 2: Perisoru, Braila County, Romania (Marinescu <i>et al.</i> , 2011)		
Parameter	Polluted	Unpolluted
pH	6.22	6.80
Organic C (%)	5.51	2.23
Total N (%)	0.313	0.200
P (mgkg <sup>-1</sup> )	73.0	3.2
K (mgkg <sup>-1</sup> )	222	206

### 3. Hydrocarbon-polluted soils and crop development

Crops grow on soils that have the right amount and proportion of nutrients, water, oxygen, and organisms. PHCs alter the fertility status of soils and hence reduce their ability to support proper crop growth and development (Abii and Nwosu, 2009).

As shown in Table 1, PHC-polluted soils are characterized by low pH. Acidic soils resulting from this pollution may cause yellowing of leaves and stunting of crops. Alkaline soil nutrients such as calcium and magnesium are also greatly reduced in acidic soils. In addition, crops grown on soils with adverse soil pH may be prone to diseases. Reduced soil pH caused by the presence of PHC in soils also favours the availability of heavy metals which may be absorbed by crops growing on these soil and this can be toxic to them (McBride, 1994) (Table 2). Though some of these metals are essential for plant growth, they become harmful when present in large quantities. Similarly, reduction in some essential plant nutrients such as nitrogen and phosphorus in PHC-polluted soil (Obire and Nwaubete, 2002; Akpoveta *et al.*, 2011) may affect proper crop development on these soils.

The increased amount of carbon in PHC-polluted soil increases the growth of PHC-oxidizing microorganisms. Though these microorganisms are beneficial for crop growth, the reverse is the case when they are present in large quantities as they compete with crops for available soil nutrients hence indirectly contributing to a reduction in crop growth (Kaye and Hart, 1997; Xu and Johnson, 1997; Tiquia *et al.*, 2002; Trofimov and Rozanova, 2003).

Activities of burrowing soil organisms such as earthworms are greatly affected in PHC-polluted soils. Earthworms contribute to soil fertility by converting organic materials into humus – the fraction of soil which is rich in nutrients needed for crop growth. The greater the humus content of a soil, the greater its ability to produce quality crops in large quantities. Earthworms are aerobic organisms, thus they perform better in soils that are well aerated. Hence, in PHC-polluted soils where there is reduced or total absence of oxygen, earthworms activity is adversely affected resulting to poor crop development.

Although increases in soil structural stability resulting from PHC pollution have been recorded (McGill *et al.*, 1981; Certini, 2005), reduced soil WHC and hydraulic conductivity have also been recorded (Trofimov and Rozanova, 2003; Suleimanov *et al.*, 2005). Since

water is vital for proper crop development, the quality and quantity of crops grown on PHC-polluted soils may be adversely affected. Odugwu and Onianwa (1987) argued that PHC-polluted soils hindered seed germination. This could have been because the hydrophobic nature of these soils reduce/prevent the availability of water and oxygen which are essential for seed germination (Adam and Duncan, 2002). Similarly, Rahbar *et al.*, (2012) reported a reduction in leaf area and root length in sunflower (*Helianthus annuus*) growing on a PHC-polluted soil. The authors attributed this reduction to water deficit induced by the presence of PHC in this soil.

It is important to note that the deeper the roots of crops, the greater their tolerance to PHC pollution especially when the pollution is within the topsoil. PHCs may also have a positive effect on crops, as they are known to stimulate crop growth when present in low concentrations (McGill *et al.*, 1981). Furthermore, Nicolotti and Eghi (1998) are of the view that these pollutants become lethal to crops only when they come into direct contact with crop tissues.

**Table 2:** Heavy metal concentrations (mgkg<sup>-1</sup>) of crops grown on crude oil polluted and unpolluted soil

Plant		Heavy Metal					
		Mn	Fe	Mo	Cd	Pb	V
<i>Urena lobata</i>		195.29	73.00	0.28	0.23	0.51	0.28
	Unpolluted	120.20	48.54	0.12	0.15	0.48	0.15
<i>Diodia scandens</i>		134.37	45.96	0.16	0.17	0.23	0.07
	Unpolluted	77.73	30.37	0.03	0.05	0.17	0.07
<i>Selaginella myoscur</i>		155.83	64.96	0.23	0.12	0.28	0.09
	Unpolluted	106.21	39.82	0.07	0.11	0.25	0.11

Source: Essiet *et al.* (2010)

#### 4. Bioremediation of PHC-polluted soils

Bioremediation involves the use of organisms for the treatment of polluted soils. These organisms which could be microorganisms or green plants eliminate, attenuate or transform the harmful substances via biological processes. Bioremediation occurs naturally (even though it could be enhanced by a number of processes), thus it is widely accepted by the general public as a safe way of treating polluted soils. The by-product of bioremediation, mainly water, CO<sub>2</sub> and cell biomass are harmless and are useful for plant growth. Bioremediation is a low cost method of treating polluted soil and does not require sophisticated techniques for its operation. It can equally be carried out *in situ*, thus eliminating the risks associated with transporting the polluted soils.

In spite of the above advantages, bioremediation is not without its shortcomings. It is regarded as a slow method of remediation. In other words, bioremediation is accomplished within a relatively long time frame compared to other remediation techniques such as chemical remediation. Some researchers have also argued that bioremediation is not suitable for the treatment of heavy metals and some high aromatic hydrocarbons which are not readily biodegraded (Kumar *et al.*, 2010). However, this mainly applies to the use of microorganisms as a wide range of plants have successfully been used for the treatment of these pollutants (Ghosh and Singh, 2005). Furthermore, microbial degradation of some pollutants may lead to

the formation of compounds which are more harmful to the environment; a typical example is the anaerobic biodegradation of trichloroethylene to vinyl chloride (Kleopfer *et al.*, 1985).

Bioremediation can be grouped into two categories depending on how and where the pollutants are treated. These categories are *in situ* and *ex situ* bioremediation. *In situ* bioremediation involves the treatment of polluted soils at the site of pollution, i.e. the polluted soil is not excavated and transported elsewhere for treatment. Treatment is limited to the depth of the soil in which the treatments can be effectively applied (Pal *et al.*, 2010). *In situ* bioremediation is achieved at lower cost with minimal disturbance to the soil; hence it is the most desirable method of bioremediation. It can equally be used for the treatment of groundwater. The basic types of *in situ* bioremediation include: **(a) Bioventing**: This involves supplying nutrients (mostly nitrogen and phosphorus) and oxygen through wells to polluted soils below the ground surface in order to stimulate the activities of the indigenous degrading bacteria. It is the most common type of *in situ* treatment and is used when the pollutants are located deep within the soil. It has been used successfully for the remediation of PHC-polluted soils (FRTR, 2005; USEPA, 2005). **(b) Biosparging**: This involves the injection of oxygen (using small diameter air injection points) below the groundwater table to stimulate the microbial degradation of organic pollutants by naturally occurring aerobic bacteria. The basic factors that may limit the effectiveness of biosparging are permeability of the polluted soil and biodegradability of the pollutants (USEPA, 1994).

*Ex situ* bioremediation involves the excavation and transportation of the polluted soil to a different location for subsequent treatment of the pollutants. In this method, there is high risk of exposure to the pollutants during the process of excavation and transportation. Examples of *ex situ* treatments include: **(a) Landfarming**: In this method, the excavated soil is spread on prepared beds and is periodically tilled so as to stimulate degradation of the pollutants by the indigenous microbes present in the soil. Landfarming is a common practice because of its low maintenance cost in addition to its clean-up ability. **(b) Composting**: This involves addition of organic amendments such as manure or agricultural waste to polluted soils so as to stimulate degradation by the indigenous microorganisms. The addition of the amendments (compost) increases the microbial population and elevates the soil temperature thus, enhancing the degradation process. **(c) Biopiles**: This technique is a hybrid of landfarming and composting. It is a modification of landfarming that involves the construction of aerated composted piles to control loss of the pollutants via leaching and volatilization. It is used mainly for the treatment of surface soil polluted with PHC. **(d) Bioreactors**: This involves the use of engineering vessels (slurry bioreactors) in the treatment of polluted soil (and water). In this method, the polluted soil is made into an aqueous suspension and mechanical or pneumatic mixing is provided inside the bioreactor /vessel (Robles-Gonzalez *et al.*, 2008). The mixing process increases the contact between the microorganism (indigenous and/or introduced) and the pollutants thus, increasing the rate of pollutant degradation. The rate of degradation depends mainly on the degradation activity of the microbes involved in the system. Environmental parameters such as temperature and pH are easily controlled in a bioreactor thus increasing the rate and extent of bioremediation. However, this method requires high construction and maintenance cost compared to other bioremediation techniques.

#### 4.1 Use of microorganisms for soil remediation

Various soil microorganisms have great potential for bioremediation. They degrade organic pollutants by using them as their carbon and energy source. Most of these microorganisms are the aerobic bacteria such as *Pseudomonas*, *Mycobacterium*, *Rhodococcus*, *Arthobacter*, *Acinetobacter*, *Nocardia* and *Bacillus* (Kanaly and Harayama, 2000; Chaillan *et al.*, 2004).

Some fungi also have the ability to degrade organic pollutants. For instance, white rot fungus (*Planerochaete chrysosporium*) is an example of ligninolytic fungi capable of degrading polyaromatic hydrocarbons and other harmful environmental pollutants (Pal *et al.*, 2010). *Cunninghamella echinulata* and mycorrhizal fungi have also been used for the remediation of PHC-polluted soil (Alarcón *et al.*, 2008). For bioremediation to be effective there must be contact between the microorganisms and the pollutants and since various types of pollutants exist in a PHC-polluted soil, a wide range of microorganisms is required for effective bioremediation.

When a polluted soil is allowed to recover on its own without any artificial aid, the soil is said to be remediated through the process of natural attenuation/monitored natural attenuation (MNA). MNA is very effective on soils with high native microbial population (Sarkar *et al.*, 2005). It is considered efficient only when the treatment is achieved within a reasonable time frame compared to other remediation techniques. Bioremediation can be achieved through the process of biostimulation which works on the principle that microorganisms responsible for degrading pollutants already exists in the soil. Therefore, conditions that promote their activities are made available in order to enhance bioremediation. This could be achieved through addition of nutrients in the form of organic manure or other organic materials or through alteration of the soil's pH, moisture or aeration status. The species and population of microorganisms present in a PHC-polluted soil determines to a large extent the effectiveness of biostimulation as a bioremediation technique. Biostimulation is faster and more effective on soils that have been previously polluted and treated than on soils that have never been polluted. This is because the population of PHC-degrading microorganisms would be greater on soils that have been previously polluted. Another important fact about biostimulation is that when nutrients are added to a soil in order to stimulate the activities of biodegrading microorganisms, the nutrients will be used by all microorganisms (that require the nutrient as their energy source) present in the soil regardless of whether they are biodegrading or not. Therefore, there is no guarantee that the nutrients will be used specifically by the biodegrading microorganisms (Naide *et al.*, 2010). However, studies have shown that biostimulation can be an effective technique for the remediation of PHC-polluted soils (Peressutti *et al.*, 2003; Bento *et al.*, 2004; Sarkar *et al.*, 2005).

Bioremediation can also be achieved through the process of bioaugmentation. In bioaugmentation, the polluted site is inoculated with a consortium of microorganisms capable of degrading the pollutants. Sometimes, genetically modified organisms (GMOs) are used. However use of GMOs for bioremediation is limited due to the controversies surrounding GMOs (Ang *et al.*, 2005; Urgun-Demirtas *et al.*, 2006). Before inoculation of the polluted site, a biocompatibility test is carried out to check for compatibility between the microorganisms and the environment. Once the microorganisms are introduced into the site, they adapt to the new environment and begin degrading the pollutant. The speed with which the pollutants are degraded depends on the concentration of microorganisms in the inoculum as well as the pollutant concentration. Bioaugmentation achieves clean-up of polluted site within a very short time frame. However, it requires higher technological expertise compared to biostimulation and may not be cost effective on smaller sites.

Studies have shown that bioremediation of PHC-polluted soil increases crop growth and development. Adedokun and Ataga (2007) reported a significant improvement in the growth of cowpea (*Vigna unguiculata*) when a soil polluted with crude oil, automotive gasoline oil and spent engine oil was biostimulated (with saw dust and waste cotton) and bioaugmented (with *Pleurotus pulmonarium*). They reported that bioremediation of polluted soil reduced the

time for seed germination from 8 to 3 days, increased seed germination from 60% to 96%, plant height from 10.3cm to 22cm, leaf number from 3 to 5 and biomass from 0.5g to 1.5g dry weight. A similar study by Kyung-Hwa *et al.* (2004) showed that the length of corn (*Zea mays*) and red beans (*Phaseolus nipponesis*) grown on a polycyclic aromatic hydrocarbon (PAH)- polluted soil inoculated with *Norcardia* spp. for bioremediation purposes increased by 77% and 56% respectively with respect to the control (unpolluted soil). However, on the polluted soils that were not bioremediated, length of corn and red beans were 16% and 49% respectively. The authors also reported that bioremediation reduced phytotoxicity in the test crops. Njoku *et al.* (2008) reported a general improvement in the chlorophyll content, leaf area, growth, pod production and dry weight of soybean (*Glycine max*) grown on a crude oil polluted soil bioremediated with cow dung. Significant increases in the germination, growth and productivity of soybean were also recorded on a crude oil polluted soil subjected to bioaugmentation and biostimulation using *Pseudomonas*, *Bacillus* and poultry manure (Nwadinigwe and Onyeidu, 2012). The study also showed that bioaugmentation performed better than biostimulation with respect to crop growth and development.

#### 4.2 Use of plants for soil remediation (Phytoremediation)

This is a type of *in situ* bioremediation that uses green plants to stabilize, extract or degrade pollutants from polluted soils. It is used mostly for the treatment of heavy metals in agricultural soils. However, it can also be used to remediate organic pollutants such as PAHs and toxic PHC. The mechanism and efficiency of this method depend on the type of pollutant, bioavailability of the pollutant, plant used and soil properties (Cunningham and Ow, 1996; USEPA, 2012). It works best when the pollutants are within the root zone of the soil. Plants assist in the remediation of polluted soil via different mechanisms namely:

1. **Phytostabilization/Phytoimmobilization:** In this method, plants reduce the mobility and bioavailability of pollutants in soils by adsorbing the pollutants into their structure. Leachable constituents are bound by the plant's structure so that they do not re-enter into the environment. It is useful when the concentration of pollutants is relatively low. Pollutants treated via this method are heavy metals and hydrophobic organic compounds such as aromatic compounds in petroleum.
2. **Phytoextraction/Phytoaccumulation:** In this method, plants extract the pollutants from the soil by accumulating them into their roots and shoots so that the plants can later be harvested and incinerated. It is used mostly for the remediation of heavy metals in soils.
3. **Phytodegradation/Phytotransformation:** Here the pollutants are degraded by compounds produced or exuded by plants. It can be used for the remediation of soils polluted with polar organic pollutants such as atrazine as well as those polluted with non-polar organic pollutants such as phenanthrene.
4. **Phytovolatilization:** This is a technique whereby plants adsorb and release the pollutants or their metabolites into the atmosphere (Palmroth, 2006; Pal *et al.*, 2010). Soils polluted with organic and inorganic pollutants (but mainly organic pollutants such as trichlorethylene) can be remediated via this method.
5. **Rhizodegradation/Phytostimulation:** This involves the breakdown of the pollutants into less toxic forms by microorganisms within the root zone of plants. It is a symbiotic relationship whereby the plants provide nutrients for the microorganisms, while, the microorganisms provide a healthy environment for the plants' growth and

development. It is used mostly for the removal of organic pollutants such as PHCs and PAHs from soils.

Most times, grasses and legumes are used for the remediation of PHC-polluted soils because their root systems provide a large rhizosphere zone (Aprill and Sims, 1990; Adam and Duncan, 1999; Merkl *et al.*, 2005). Some examples of grasses that have successfully been used for remediation of PHC-polluted soils include guinea grass, signal grass, prairie grasses, and rye grass (Aprill and Sims, 1990; Guenther *et al.*, 1996; McCutcheon and Schnoor, 2003). Some legumes used for the same purpose include alfalfa, sorghum, red and white clover (Schwab *et al.*, 1995; McCutcheon and Schnoor, 2003). These plants reduce the concentration of total PHCs and/or PAHs in soils using one or more of the mechanisms described above. Some other crops such as maize and sunflower have also been used for the remediation of PHC-polluted soils (Chaineau *et al.*, 2000; McCutcheon and Schnoor, 2003). These crops are able to degrade PHC present in soils. Ogbo *et al.* (2009) reported that weeds such as *Sida rhombifolia* and *Mariscus alternifolius* reduce about 60% of saturated hydrocarbons when used for phytoremediation of soils polluted with spent lubricating oil.

Plants used for phytoremediation have specific tolerance limits. Rahbar, *et al.* (2012) noted that sunflower can thrive in a soil containing about 18,000mg/kg of PHC by alterations in metabolic activities that counter the destructive effect of the pollutant. A good method of identifying plants that can be used for phytoremediation is to record the species that naturally thrive on polluted soils. Major traits exhibited by phytoremediators include extensive root system, ability to either degrade or concentrate the pollutants at high levels in the biomass, ability to absorb large amounts of water from the soil, fast growth rate and high levels of biomass (ISAAA, 2006).

Treatment of polluted soils via phytoremediation takes a long time and there may be contamination of the vegetation and food chain through phytoremediation activities if proper care is not taken. However, phytoremediation is desirable when the polluted site is so large that other methods of remediation are not cost effective. It is also desirable when the pollutants are at low concentrations that only polish treatments are required over long periods of time. Finally, it can be used in conjunction with other methods of remediation whereby the plants are used for final site closure (Pal *et al.*, 2010).

#### **4.3 Combining microorganisms and plants for soil remediation: Mycorrhiza-assisted remediation**

Although plants can be used for the treatment of soils polluted with organic pollutants, high concentrations of these pollutants may be harmful to plants thus leading to reduced remediation (Germaine *et al.*, 2009). Inoculating phytoremediators with suitable microorganisms reduces the concentration of pollutants through increased degradation by the added microorganisms. The inoculated microorganisms also increase the growth of plants - by alleviating plant stress - thus making them more suitable for the remediation process (Weyens *et al.*, 2009). Improved soil remediation has been reported following inoculation of phytoremediators with suitable microorganisms (Weyens *et al.*, 2009; Glick, 2010).

One group of soil microorganisms that have been widely used for bioremediation of polluted soils is soil bacteria. Both rhizobacteria and endophytic bacteria such as *Azospirillum* spp. and *Pseudomonas* spp. have been used in conjunction with plants such as wheat and pea for the remediation of PHC-polluted soils (Shaw and Burns, 2004; Muratova *et al.*, 2005; Germaine *et al.*, 2009). Some fungi such as *Cunninghamella echinulata*, *Fusarium* spp. and

*Altenaria* spp. have also been used together with plants for the remediation of PHC-polluted soils (Alarcón *et al.*, 2008; Mohsenzadeh *et al.*, 2010). Another important group of soil fungi which have been explored for their soil remediating ability is the mycorrhizal fungi. Mycorrhiza is the symbiotic association between mycorrhizal fungi and the roots of vascular plants. The plant supplies carbohydrates to the fungi while the fungi increase the ability of the plant to absorb nutrient (especially phosphorus) and water from the soil. The fungi also increase the plant's ability to withstand diseases (Harrier and Watson, 2004). A certain group of mycorrhizal fungi known as arbuscular mycorrhizal fungi (AMF) are able to improve the structure of the soil through the production of glomalin – a glycoprotein which binds soil aggregates thus making the soil fit for crop production (Wright *et al.*, 2007).

Mycorrhizal fungi are widely known for their ability to increase plant tolerance to heavy metals in polluted soils (Wasserman *et al.*, 1987; Smith and Read, 1997; Turgay *et al.*, 2009). Bioremediation of polluted soils with plants inoculated with mycorrhizal fungi is known to be faster and more efficient than ordinary phytoremediation (Alarcón *et al.*, 2008; Gao *et al.*, 2010). Gao *et al.* (2010) attributed this enhanced remediation to the larger area covered by the mycorrhizal plant roots. The mechanism employed by mycorrhiza for the remediation of polluted soils is not well understood. However, the works of Binet *et al.* (2000), Alarcón *et al.* (2008) and Gao *et al.* (2010) show that biodegradation, phytoextraction and phytostabilization are possible mechanisms used by this association for the remediation of crude oil and PAH-polluted soils.

AMF may directly affect PAH degradation by enhancing the production of extracellular peroxidases (Leyval *et al.*, 2002). It may also affect PAH (and other PHCs) degradation indirectly by modifying the structure of the associated microbial communities (Cairney and Meharg, 2002; Leyval *et al.*, 2002). These modified microbial communities may include other microorganisms that not only assist in further degradation of the pollutants but may also be antagonistic to harmful soil organisms (Harrier and Watson, 2004). This in turn makes the soil free of these harmful organisms, thus making it more suitable for crop production.

Mycorrhizal fungi help in the uptake of water and water-dissolved inorganic nutrients in soils rendered hydrophobic due to PHC contamination (Leyval and Binet, 1998). Therefore, the fungi not only assist in soil remediation but also help phytoremediators survive in the hydrophobic environment. Parrish *et al.* (2005) reported that reduction of PHC toxicity is more rapid in soils containing plants and mycorrhizal associations.

Trees or other crops (from uncontaminated sites) inoculated with mycorrhizal fungi may be planted (or transplanted) on polluted soils so as to achieve final soil clean-up (Fraser and Kindscher, 2005). Although it may take a long time for the mycorrhizal biomass to establish, the fungi mycelia remain in the soil for several decades, remediating the soil and providing conditions for easy re-vegetation of these sites. It is essential to have a prior knowledge of the fungi symbionts likely to survive in an ecosystem before adoption of this technique. It is also advisable to know if the fungi to be used would contribute directly or indirectly to PHC degradation before they are used for the remediation exercise (Robertson *et al.*, 2007).

## 5. Conclusion

Bioremediation of PHC-polluted soils generally restores the biological as well as the physicochemical properties of degraded soils thus making them fit for crop production. Although microorganisms and plants can be used independently for the clean-up of polluted sites, combining these two groups of organisms (microorganisms and phytoremediators)

increases the efficiency of this method of remediation. There is need for more research on the various microorganisms and plants that could be combined for remediation of PHC-polluted soils. An understanding of the plant-microorganism-soil interaction in polluted environments is also essential for effective bioremediation.

## 6. References

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