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Bioremediation of a Soil Contaminated with Lubricating Oil using Bacteria Consortium

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Abstract: A pilot study was carried out on soil from toll gate area in Ibadan, Oyo state western Nigeria, contaminated with hydrocarbon (lubricating oil) by artificial simulation to determine the attendant effect associated with the soil physicochemical properties and microbiological composition. Biodegradation of the contaminant using soil microbes and the kinetics of such process was also investigated. Soil parameters such as pH, conductivity, total organic hydrogen, total nitrogen and phosphorus and total petroleum hydrocarbon (TPH) were characterized using standard analytical methods. Trend in growth phase of soil heterotrophic and hydrocarbon utilizing microbes were investigated. Hydrocarbon contamination was seen to affect certain soil properties as a reduction in pH, conductivity, total phosphorus and heterotrophic microbial population was observed. The rate of microbial degradation was found to be dependent on pH and nutrient source. Effective degradation and increased microbial growth occurred between pH 5.3 and 7.2 but recorded reduced microbial growth and rate at much higher pH, thereby defining a suitable pH condition for the process.
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1. Introduction

Bioremediation is the use of microorganism metabolism to remove pollutants. Bioremediation technologies can be generally classified as *in situ* or *ex situ*. *in situ* bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some examples of current bioremediation technologies include; phytoremediation, bioventing, bioleaching, land-farming, bioreactor, compositing, bioaugmentation, rhizofiltration and biostimulation (Busetti, 2005).

Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators (Akpoveta and Osakwe, 2010).

However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. The assimilation of metals such as mercury into the food chain may worsen matters (Bergey and Breed, 1997). Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use (Mills et al., 1998).

There are recently global concerns over soils contaminated with crude oil or hydrocarbon products in general, after a similar feeling has been around for a while on marine oil-spills, which enjoy more media coverage because of the often spectacular visual effects images conveyed to people (Al-Mailem et al., 2010). There are similarities and differences between inland and offshore crude oil-spills. Similarities include hazards to life in all its forms. Secondly, contamination of valuable fresh water resources from aquifers or desalination plants and long term environmental impact; despite unsubstantiated claims that nature fully recovers in a few years. On the other hand, the differences concern mainly the behavior of spilled oil, its interaction with the surrounding environment and the corresponding approach to remediation (Bamnger et al., 2005).

In the case of soils contaminated by hydrocarbon products, there has been a great deal of work on biologically based treatment processes from several disciplines of the scientific community (Duii et al., 2002). This is not an odd phenomenon since

environmental research concerns just as many disciplines and more importantly attracts funding support from government and private sources. However, the diversity of backgrounds of the researchers created a collection of schools of thought as well as, sometimes convenient basis for agreement or disagreement in interpretation of laboratory or field data on bioremediation (Barrir et al., 2006).

The aim of this study is therefore, to investigate the bioremediation activity of bacteria consortium on a hydrocarbon polluted soil and the kinetics involved in the process.

2. Materials and Methods

Soil samples were obtained from Lead City University premises. The lubricating oil was purchased at mobil filling station Toll gate Ibadan. *S. saprophiticus*, *S. aureus*, *P. aeruginosa*, *E. coli* and Klebs were obtained from the microbiology laboratory of University College Hospital, Ibadan, Nigeria.

2.1 Soil Preparation and Sampling

A representative sample of the soil to be used was collected, dried and sieved using a wire mesh of 2mm. 20g of soil was weighed into five 250ml beakers and the samples were labeled A, B, C, D and E. Samples B,C and D were sterilized by placing it in hot air oven at 180°C and weighed at interval. The sterilization process was completed when the weight remains constant.

2.2 Preparation of Microbial Culture

The bioremediator was made up of an oil-degrading bacteria consortium containing *Staphilococcus saprophiticus, Staphilococcus aureus, Pseudomonas aeruginosa, E. coli* and Klebs. These were previously isolated and sub-cultured using nutrient agar medium. The medium was prepared by first weighing 6.2g of nutrient agar concentrate (with original concentration of 31g/l) and dissolving it in 200ml of distilled water (Chiu et al., 2000 and Dave, 2010). Thereafter, the solution was homogenized by boiling it in a water bath. After homogenizing, the medium was sterilized by autoclaving at a temperature of 121°C for 30minutes. It was allowed to cool for about 30 minutes (during the cooling process, the medium was swirled continuously to avoid solidification). The medium was poured into Mc Artney's bottle and the bottles were left in a slanted position until the medium solidifies (Grassi and Netti, 2000). Using an inoculating needle which has been pre-sterilized by flaming it on the methylated lamp, an inoculum was picked from the original culture and streaked on the surface of the prepared slants. The new isolates were stored in the incubator at 40°C and allowed to grow for 48 hours (Mokolobate and Haynes, 2002a).

2.3 Harvesting

The new culture was obtained from the incubator; about 15ml of peptone water was added into the bottles containing the culture. Using inoculating needle, the microbial cultured was streaked off into the water. Peptone water was used in this case to provide nutrient for the microbial culture (Ramalhosa et al., 2000, Khan et al., 2005 and Olipdri et al., 2009). The solution was then transferred into the contaminated soil sample.

2.4 Experimental Design

20g of sieved soil, which has been thoroughly mixed together was weighed into five 250ml beakers, the beakers were labeled A, B, C, D and E. Four of the samples were contaminated by adding 15ml of lubricating oil. Test carried out on each of the samples is as follows:

Sample A contains unsterilized soil and lubricating oil, this sample was used to monitor the action of the indigenous bacteria on the oil (Bouyouces, 1991). Sample B contains sterilized soil and bacteria consortium; this sample was used to monitor the effect of the introduced bacteria on the uncontaminated soil. Sample C contains sterilized soil and oil; this sample acts as the control (no microbes either foreign or indigenous). Sample D contains sterilized soil, lubricating oil and bacteria consortium; this sample was used to monitor the action of the introduced bacteria on the contaminated soil. Sample E contains unsterilized soil, bacteria consortium and lubricating oil; this sample was used to monitor the effect of combined microbes (both foreign and indigenous) on the contaminated soil (Bray and Kurtz, 1993). The soil samples were incubated for 60 days, after which they were subjected to the following analysis; soil pH, conductivity, Total Petroleum Hydrocarbon (TPH), Polyaromatic Hydrocarbon (PAH) and elemental constituents i.e. hydrogen, nitrogen, sulphur and phosphorus (APHA, 1998). The values were expressed as Mean ± Standard deviation.

3. Results

The physico-chemical characteristic of the soil influenced by the impact of lubricating oil is shown in tables below:

Table 1. Physico-chemical properties of soil at 60th day of study

Samples	pH	Cond uctivity (µS/cm)	TPH (mg/kg)	Phosphate (mg/kg)	Hydrogen (mg/kg)	% Nitrogen	Sulphate
A	5.43	2000	1153.13	111.87	10.54	0.015	Nd
B	6.91	2720	277.78	149.15	0.004	0.0073	Nd
C	5.31	3990	4333.33	82.17	7.32	0.013	Nd
D	5.90	2590	45833.33	9.14	5.78	0.032	Nd
E	6.27	2170	38555.56	123.69	9.76	0.035	Nd

A – Unsterilized Soil + Oil
B – Sterilized Soil + Bacteria Consortium
C – Sterilized Soil + Oil
D – Sterilized Soil + Oil + Bacteria Consortium
E – Unsterilized Soil + Oil + Bacteria Consortium

Table 2. Concentration of heavy metals in soil before and after contamination

Concentration (l/kg soil)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Pb (ppm)
0_{BP}	3.57	3.22	1.36	0.29
0_{AH}	25.50	3.50	1.85	0.33
0.2	83.50	4.90	1.98	0.58
0.4	134.80	7.30	2.13	0.55
0.6	228.40	8.64	2.59	0.75
0.8	301.00	12.10	2.81	0.81

4. Discussions

The physicochemical characteristics of the soil were influenced by the impact of hydrocarbon contamination as observed in table 1 above. A reduction in pH, increase in conductivity and total phosphorus were observed on simulation of the soil with hydrocarbon (lubricating oil) from 7.2 to 5.3, 1891FS/cm to 3990FS/cm and 2.7mg/kg to 4.5mg/kg respectively; while a significant increase in total petroleum hydrocarbon (TPH) from 8.64mg/kg in the control soil to 1894.87mg/kg in the lubricating oil simulated soil was recorded as seen in the table (Dimitrow and Markow, 2000). The weak acidity observed in the control soil is common with reduced anaerobic soils and sediments in the Niger Delta (Mokolobate and Haynes, 2002a and Maletić et al., 2009). The pH for the unpolluted soil fell within the pH range of between 5-7 which is suitable for most good agricultural soils, since Osuji et al., (2005) reported that most good agricultural soils have a pH between 5 and 7. Increased acidity occasioned by the presence of hydrocarbon (lubricating oil) is a problem for agricultural soil because very low pH values, indicative of acidity, are associated with adverse soil conditions including reduced microbial activity, increased availability and toxicity of heavy metals as well as reduced availability of plant nutrients. Conductivity value recorded in the control soil is due to the presence of soluble polar mobile solutes in the soil. The resulting decrease on contamination is due to the effect of hydrocarbon (lubricating oil) which provides a non polar environment for the soil ions, retarding their movement and immobilizing them, resulting in reduced ionic mobility, velocity and consequently bringing about increased conductivity. Presence of hydrocarbon in soil reduces available forms of phosphorus as has been shown by Okiemen and Okiemen (2005) and Okonokhua *et al*., (2007). The observed reduction in pH and increased conductivity was similar to the findings of Osuji and Nwoye (2007). After the bioremediation process, a decrease in pH (7.2 to 5.3), increased conductivity (1891 to 3990FS/cm) and total phosphorus (2.7to 4.5mg/kg) were observed. Substantial reduction in hydrocarbon concentration thereby providing a polar environment for the soil ions accounted for the increased conductivity. Introduction of exogenous nutrients such as phosphorus, nitrogen and other cat ions from the animal waste used in the bioremediation process possibly explains the observed increase in pH and total phosphorus content. Soil properties such as total nitrogen (0.007 to 0.15 to 0.35mg/kg), and organic phosphate (9.1 to 82.1 to 149.) increased on addition of the hydrocarbon to the soil and subsequently increased after the bioremediation process.

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