# BIOREMEDIATION OF HYDROCARBON POLLUTION: A SUSTAINABLE MEANS OF BIODIVERSITY CONSERVATION

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**Abstract**

**Hydrocarbon pollution of the natural environments poses enormous danger to wide variety of species of living organisms. This disrupts interrelationships that exist between them and eventually serves as immediate cause of death. For example, Crude oil can impair egg formation, egg laying, incubation and well being of birds and fish. In addition oil can cause mortality and reduced hatching of eggs. Also when insects are smothered with crude oil, they lose their flying ability and eventually die of suffocation and elevated temperatures. These profound effects made it a source of concern considering the dispersal and pollination role the organisms play. Another consequence is also that of species extinction and gene reduction. Bioremediation experiment was carried out to assess the biodegradative capacity of bacteria on Bonny light crude oil. This was with a view to reducing the level of toxic components of Bonny light crude oil. Selective enrichment technique was used to isolate bacteria that have a chemical appetite for crude oil and the same bacteria were used in degradation experiment. When the bacterial consortia was inoculated into mineral salt medium overlaid with Bonny light crude oil as the only carbon source, transformation of some oil components took place. This was evident by the results of chromatographic analyses generated using GC-MS. The Polycyclic aromatic hydrocarbons were significantly ($P<0.05$) reduced suggesting a reduced impact on biodiversity.**

**Keywords: Bioremediation, Biodiversity, hydrocarbon, crude oil and sustainability**

## INTRODUCTION

**All ecosystems and human societies depend on healthy and productive natural environments that contain diverse plants and animal species. The rapidly growing world population and increased human activity threaten many of these species. Incidentally some of these species of living organisms contribute a number of economic benefits to man and the environment. For example, Crude oil formation, soil formation, waste disposal, nitrogen fixation, biological pest control, pollination, dispersal of fruits and production of pharmaceuticals can all be accomplished through the exploitation of enormous biodiversity (Thorpe *et al. 1995*; Farnsworth and Soejarto, 1997). Pollinators such as insects, birds and bats provide substantial benefits to the maintainance, diversity and productivity of both agricultural and natural ecosystems (Buchman and Nabhan, 1996). As much as one third of the world's food production relies either directly or indirectly on insect pollination (Fujita and Tuttle, 1991; Richards, 1993). Pollinator diversity depends on ecosystems that are rich in diverse vegetation. Unfortunately hydrocarbon contamination of agricultural lands that results from oil spillage kills vegetation and hence the biodiversity associated with it.**

**Hydrocarbon substances particularly the polycyclic aromatic types have a deleterious effect on biota. Poly aromatic Hydrocarbons can build up in living tissues and so the PAH contents of plants, invertebrates and fish can be many times higher than the content of PAHs in soil and water. Bioconcentration**

factors (BCF) which express the concentration in the tissues compared to the concentration in water for fish and crustaceans are frequently in the 10-10,000 range, although a BCF of 134,000 has been reported for BaP in water flea (*Daphnia pulex*). In water PAHs do not generally dissolve easily but tend to stick to a particulate matter. PAHs stuck to small particles may be found in surface micro layer, but those stuck to larger particles will settle out to sediments. In soil and water, breakdown generally takes weeks to months and the action of microorganisms is usually primarily responsible. In the air, PAHs can be carried long distances stuck to the surfaces of small solid particles and even remote ecosystems have been contaminated.

Poly aromatic Hydrocarbons return to earth in rainfall or particulate settling, or alternatively they can breakdown to longer lasting products by reacting with sunlight and other chemicals in the air over a period of days to weeks. Unfortunately, many PAHs transformation products such as nitro-PAH and hydroxyl-PAH are more carcinogenic than the parent compounds.

The effects of PAHs are mostly known from animal experiments, but because of the similarity of Biological systems in different species, it is likely that all mammals, including humans will be affected in a similar way unless if they metabolize these substances differently. It is the metabolic products of PAHs that give rise to their toxicity. PAHs cause cancer (Chaloupka *et al.,* 1993). Several PAHs including benzo (a) anthracene, benzo (a) pyrene (BaP), benzo (b) fluoranthene, chrysene, dibenzo (ah) anthracene, indeno (1,2,3-ed pyrene) have caused tumours in laboratory animals by inhalation and skin contact. Furthermore an increase in mammary tumours in rats has been caused by both a single dose of BaP (100mg/kg) and from eight weekly doses of 12.5mg/kg. Experiments with BaP, which is representative of other cancer causing PAHs show that fish seems to be most susceptible to peak exposures of BaP, particularly after earlier exposure to lower levels. This may have possibly caused induction of metabolizing enzymes required to activate the molecule (Potter et al., 1994). Coke oven workers run an increased risk of developing respiratory cancers as do other workers exposed to PAHs where those substances may also contribute to skin and bladder cancers. PAHs disrupt the sex hormones and possess reproductive and developmental toxicity. There is currently a lack of data on the reproductive and developmental effects of many individual PAHs and even for BaP the data are conflicting. Annual studies suggest effects on sperm quality, but females may be at increased risk of reproductive dysfunction because oocyte and follicular destruction can occur as a result of exposure. Since the testes and ovaries contain rapidly proliferating cells, they are probably particularly susceptible to damage by PAHs. BaP can certainly affect gg production in fish. Exposure has been found to decrease primary oocytes number and reduce plasma testosterone and oestrogen levels ( Thomas, 1990). Also experiments show that certain PAHs can be transferred to the egg from female fish, and can cause a decrease in the number of eggs laid, as well as decreased fry survival. Exposure of egg to the sun may increase the toxicity of PAHs as in addition to a decrease in the number of eggs laid and decreased fry survival. Teratogenic effects and decreased percentage hatch were also observed in the fry and eggs of the fish exposed to solar ultraviolet radiation (Hall and Oris 1991). PAHs are considered to be developmental toxicants. Limited animal data, mostly relating to the effects of BaP indicate that PAHs have a potential to induce adverse developmental effects such as pregnancy terminations, malformations, sterility in offsprings, testicular changes including wasting with lack of sperm, immunosuppression and tumours.

In adult birds exposure to crude oil can cause stress which shuts down reproduction *via* feedback to the brain. Crude oil can also impair egg formation, egg laying, incubation and stability of of the pair bond. In addition oil can cause mortality and reduced hatching of eggs with the PAHs components being the most toxic (Ronald, 1987). When the feathers of birds are soaked in crude oil, they get smothered and hence losses their ability to fly. On the other hand, when insects are affected they get incapacitated and experience a rise in physiological temperature leading to death.

In this research, bioremediation option was considered to test the effect of bacterial consortium on biodegradation of polycyclic aromatic hydrocarbons contained in Bonny light crude oil.

## MATERIALS AND METHODS

### CRUDE OIL DEGRADATION

The cultures used in the degradation experiment were maintained in crude oil – overlaid – minimal medium. Nutrient broth (10ml) was dispensed into universal bottles. Then the test bacteria were inoculated into these media and the bottles were incubated at 24°C for 48 hours. Eight millilitres of this broth culture containing the actively growing bacteria were inoculated into other sets of universal bottles containing minimal medium overlaid with Bonny light crude oil.

### DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

All chemicals and reagents used were of analytical grade. The dichloromethane used as an extractant was obtained from Fischer Scientific (UK). A Poly Aromatic Hydrocarbon mixture (NIST, Baltimore, MD) containing naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benz (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, benzo (ghi) perylene, dibenzo (a, h) anthracene and indeno ( 1, 2, 2 d) pyrene was prepared and used as standard. Also four isotopically labeled Poly Aromatic Hydrocarbons; acenaphthelene-$_{d10}$, chrysene-$_{d12}$, penanthrene-$_{d10}$ and perylene-$_{d12}$ (ChemService, Westchester, PA) were used as internal standards.

#### 3.10.1 Preparation of Standard Solutions

Five standard solutions each containing 16 target compounds were prepared by diluting the standard mix (i.e. mixture from NIST) to desired concentrations with HPLC grade dichloromethane. Then, 0.5ug of internal standard was added into these solutions. The final mixture was then transferred to a capped and sealed vial until ready for analysis.

#### 3.10.2 Extraction of Residual Oil from Degradation Bottles

The extraction of residual oil from the degradation bottles was carried out in accordance with standard procedures described in USEPA (1994). In this method, a 100ml capacity separating funnel was mounted on a retort stand. The separating funnel was thoroughly washed and dried overnight in a murfle furnace operated at 150°C. Prior to use, the funnel was rinsed vigorously with dichloromethane for several minutes. The funnel was then removed and allowed to drain completely in fumed cupboard. Then 20ml of water-oil mixture was transferred from the degradation bottles to the funnel and to this was added 20ml of dichloromethane. The mixture was shaken vigorously for four minutes and allowed to separate and settle. After ten minutes, the organic layer that formed was removed and the process repeated with the aqueous layer twice. The three portions of the organic phase were combined and evaporated to 1ml volume using a rotary evaporator. The percentage recovery was calculated thus:

$$\%R = Q_d/Q_a \times 100$$

Where:

Qd= Quantity determined by analysis and

Qa= Quantity added

### 3.10.3 Calibration

Several dilutions of the standard PAH mixtures made were analyzed to determine detection limits and limit of quantification.. For such determinations triplicate analyses were used.

### 3.10.4 Analysis by GCMS

The GCMS analyses was carried out on a Finnigan Magnum instrument equipped with a CTC A200S auto sampler and a 30um, 0.25 ID DB-5 MS fused silica capillary column (J and W Scientific, Folson CA). Helium was used as a carrier gas and a column head pressure was maintained at 10 psi to give an approximate flow rate of 1ml/minute. The injector and transfer line were maintained at 290 and 250°C respectively. All injection volumes were held at 1ul in the split less mode. The column temperature was initially held at 70°C for four minutes and ramped to 300°C at the rate of 10°C/minute. This temperature was maintained for ten minutes. The mass spectrophotometer was used in electron ionization mode and all spectra were acquired using a mass range of m/z 50-400 and automatic gain control.

### 3.10.5 Identification and Quantization of the compounds

The identification and quantization of the compounds was based on the retention time match and mass spectra match against the calibration standard. Quantitation was performed using internal standardization method. Acenaphthene -$_{d10}$ was used as the internal standard for naphthalene, acenaphthylene, acenaphthene and fluorine. Phenanthrene was used as the internal standard for phenanthrene, anthracene, fluoranthene and pyrene. Chrysene-$d_{12}$ was used for Benz(a)anthracene and chrysene. Perylene-$_{d12}$ was used for Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(g,h,i)perylene, Dibenz(a,h)anthracene and Indeno(1,2,3-cd)pyrene. The quantitation was based on the ratio of the peak height of the substance being quantified to that of the corresponding internal standard.

## RESULTS

**Table 1: Polycyclic Aromatic Hydrocarbons contents of Bonny Light Crude Before Degradation**

| Hydrocarbon Component | Concentration (mg/L) |
|---|---|
| Naphthalene | 8.77 |
| 2-Methyl naphthalene | 46.4 |
| Acenaphthylene | 67.4 |
| Acenaphthene | 91.1 |
| Fluorine | 280 |
| Phananthrene | 310 |
| Anthracene | 354 |
| Fluoranthene | 354 |
| Pyrene | 410 |
| Benzo (a) anthracene | 342 |

| | |
|---|---|
| Chrysene | 302 |
| Benzo (b) fluoranthene | 416 |
| Benzo (k) fluoranthene | 322 |
| Benzo (a) pyrene | 389 |
| Dibenzo (a, h) anthracene | 419 |
| Benzo (g, h, I) Perylene | 392 |
| Indeno (1, 2, 3-d) Pyrene | 330 |
| **Total** | **4,833.67** |

**Table 2: Mean Values of Residual Polycyclic Aromatic Hydrocarbon Content (mg/L) in Bottles Seeded with different Concentrations of Cow dung and Bacteria.**

| Hydrocarbon Components (mg/kg) | Varying Concentration of Cow dung (g/L) | | |
|---|---|---|---|
| | **3.22** | **6.44** | **12.88** |
| Naphthalene | 6.34 | 3.26 | 2.22 |
| 2-Methyl naphthalene | 19.2 | 9.10 | 7.23 |
| Acenaphthylene | 131.5 | 61.95 | 45.95 |
| Acenaphthene | 82.55 | 56.45 | 32.45 |
| Fluorine | 154.4 | 43.15 | 36.80 |
| Phananthrene | 137.65 | 123.00 | 57.45 |
| Anthracene | 59.65 | 39.75 | 35.00 |
| Fluoranthene | 54.45 | 35.95 | 14.30 |
| Pyrene | 24.00 | 23.10 | 16.60 |
| Benzo (a) anthracene | 27.15 | 31.30 | 44.20 |
| Chrysene | 81.95 | 45.25 | 69.05 |
| Benzo (b) fluoranthene | 37.20 | 15.40 | 33.00 |
| Benzo (k) fluoranthene | 34.75 | 12.60 | 23.25 |
| Benzo (a) pyrene | 12.60 | 18.17 | 21.95 |
| Dibenzo (a, h) anthracene | 09.82 | 09.86 | 09.07 |
| Benzo (g, h, I) Perylene | 14.30 | 10.75 | 14.27 |
| Indeno (1, 2, 3-d) Pyrene | 03.72 | 06.95 | 03.19 |

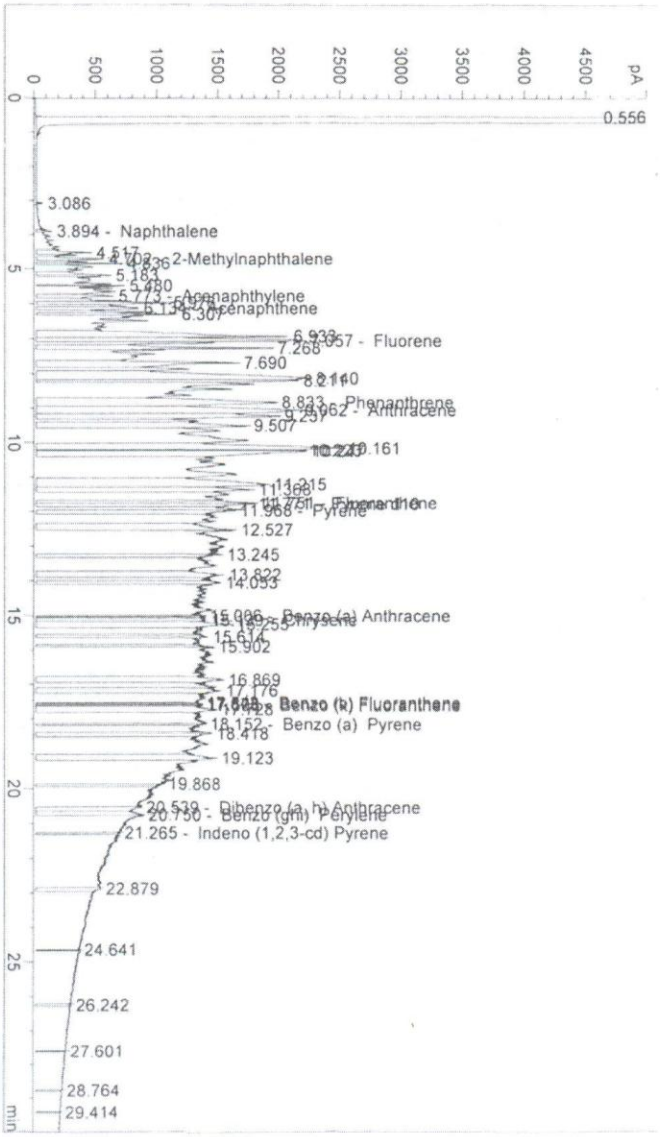


*Fig 1: Chromatogram showing the different fractions of undegraded Bonny light crude oil*

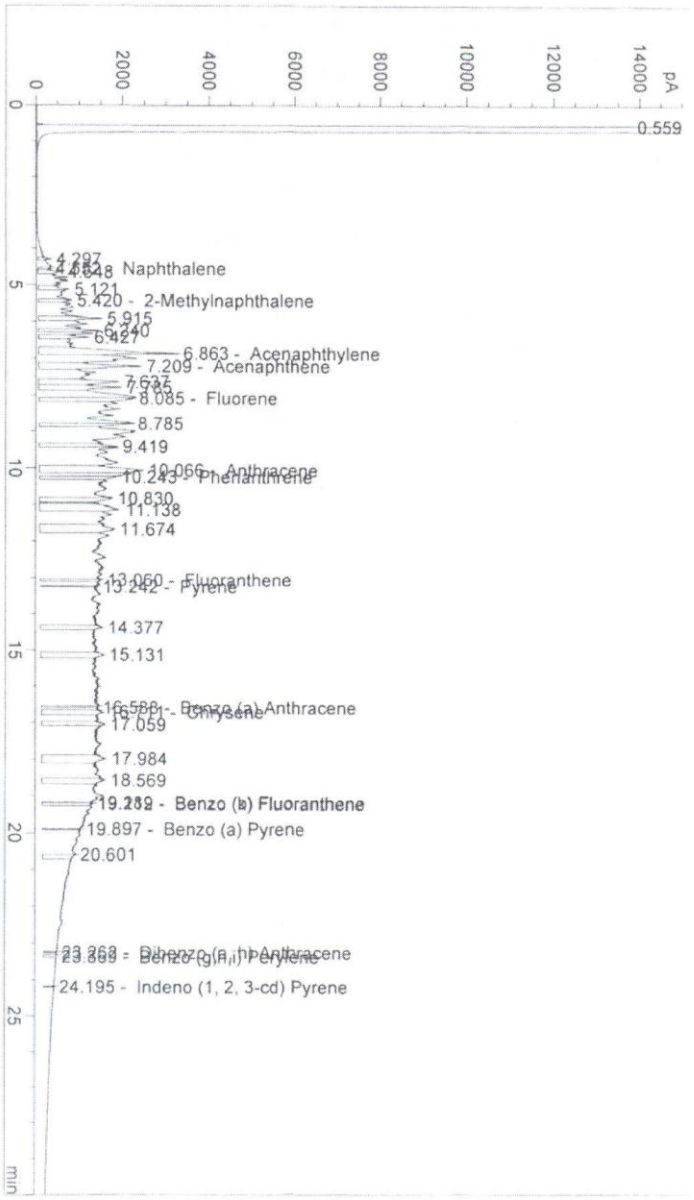


*Fig. 8: Chromatogram showing different fractions of Bonny light crude oil seeded with 3.22% of Cow dung and bacteria.*

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## DISCUSSION

Comparison between Table 1 and 2 shows a significant reduction (P<0.05) of Polycyclic Aromatic Hydrocarbons. The reduction of Polycyclic Aromatic Hydrocarbons (PAHs) using different wastes achieved in this research work is of significant importance in view of the numerous toxicity and health problems they cause. The PAHs are ubiquitous environmental contaminants. The health implications of PAHs had been reported by Thomas (1990); Hall and Oris (1991); Chaloupka (1993) and Potter (1994).

A major observation can be made from the reduction of those PAHs that are of serious health hazards. They include Benzo (a) pyrene, Benzo (a) anthracene, Chrysene, Dibenz (a,h) anthracene, Benzo (b) fluoranthene, Indeno (1,2,3 –d) pyrene and Benzo (g,h,i) perylene. It is interesting to note that in this study, all these compounds were significantly (P<0.05) degraded using the combination of bacteria and wastes used as seed. In the case of Benzo (a) anthracene, the reduction of Polycyclic Aromatic Hydrocarbons (PAHs) using different concentarations of cowdung used as biostimulants achieved in this research work is of significant importance in view of the numerous toxicity and health problems they cause.

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