

## PHYSICOCHEMICAL ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBON CONTAMINATED SOILS OF THE WESTERN RAJASTHAN

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### ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compound that are considered as ubiquitous contaminants in the environment. It deteriorates soil structure, organic matter contents and soil minerals nutrients. Almost 5 soil samples were collected from hydrocarbon contaminated areas of Western Rajasthan to be analyzed physicochemical. The physicochemical properties were evaluated using different analytical methods. Soils collected from nearby unpolluted sites were used as Reference. The results revealed that increase in PAH spillage decreased the soil pH thus making it unsuitable for the vegetation. Organic carbon increased with increase in pollution while, available potassium and phosphorus decreased with increase in acidification of the soil. High salt concentrations have lead to the high conductivity. The concentration of heavy metals increased with the increase in PAH spillage. This result revealed that the polluted soils as compared with the reference were unsuitable for agricultural activities and required an ideal Bioremediation technology.

**Keywords:** Polycyclic Aromatic Hydrocarbon (PAHs), Polluted Soil, Bioremediation, Western Rajasthan

### INTRODUCTION

Petroleum hydrocarbons, although not xenobiotics, are due to their large-scale use as one of the main potential sources of environmental contamination (Amellal *et al.*, 2001). Diesel fuel is a complex mixture of normal, branched and cyclic alkanes and aromatic compounds obtained from the middle middle-distillate, gas-oil fraction during petroleum separation (Minai and Herfatmanesh, 2007). With the growing demand for diesel by many vehicles and generators, greater quantities of diesel is being transported over long distances, leading to diesel spillages, where the components of diesel could pose serious environmental threats when it directly or indirectly enters into the environment because of their chemical nature. Since petroleum products contain some gaseous components, these fractions will volatilize when there is oil pollution leaving the non-volatile components as residues in and on the soil. It has been demonstrated that oil spillage affects the physical and chemical nature of soils (Hussein, 2012). The economic and environmental impacts of oil pollution on the soil are enormous causing serious damage to vegetation, soil fertility and soil-borne micro organisms (Kisku *et al.*, 2000).

Bioremediation of such soils involve intentional exposure of microbes to the contaminated site for clearance of the pollutants. For the optimization of effective diesel bioremediation processes it is essential to consider environmental and biological factors affecting the process. The environmental factors include availability of nutrients, pH, soil texture and extent of diesel contamination in the polluted soil, whereas the biological factors encompass the presence of bacterial, fungal or algal species which are responsible for bioremediation of diesel in the contaminated soils (Bahuguna *et al.*, 2011).

Fungi plays an important role in removing hazardous compounds from the water and soil, where as sediment particles contaminated with petroleum products from spills is one of the desired ecological to fungi which inhibits such substrate and use carbon source from hydrocarbons in polluted sediment particles to biodegrade hydrocarbons from the sediments in the beaches. Fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria (Smita *et al.*, 2012). It leads to deterioration of soil structure, loss of organic matter contents, loss of soil minerals nutrients such as potassium, sodium, sulphate, phosphate, and nitrate etc Lily *et al.*, (2009) exposing soil

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to leaching and erosion. This way causing deteriorating effects on plants indirectly by making toxic minerals present in the soil available to plants. In order to cope up with the new strategies and policies for protecting and preserving the environment we significantly require a periodical monitoring and evaluation of such contaminated sites (Sharma *et al.*, 2006). Our work aimed at assessing the deterioration in the quality of soils of the Western Rajasthan since the contamination with hydrocarbons.

## MATERIALS AND METHODS

### Description of the Sampling Sites

In view of our work, 5 sampling sites were chosen for the collection of PAH contaminated soils. These included garages, oil drilling areas, and fuel points of the railway stations, petrol pumps and oil spillage sites.

### Collection of the Soil Samples

Surface was dug up to 1cm depth and soils were collected in pre sterilized polythene bags, kept at 4°C prior to experimentation. The samples were labeled accordingly (S1-S5). A geographically similar virgin area located fifty meters adjacent to the hydrocarbon polluted area was used as control (reference) sample (R1).

### 1. Physico Chemical Analyses

pH, Conductivity, Total Organic Carbon (TOC), Available Phosphorus & Potash ( $\text{kg ha}^{-1}$ ) and Available Micronutrients elements Zinc, Iron, Copper & Manganese (ppm).

#### pH

Soil pH was determined by the method of Bates (1954), 20g of air-dried soil was taken in a 50ml beaker and 20ml distilled water was added, the contents were stirred occasionally with a glass rod and then allowed to stand for 30 minutes. Meanwhile, pH meter was calibrated. After calibration, the electrodes of the pH meter were inserted into the suspension and the pH reading was recorded.

#### Conductivity

Since ions are the carrier of electricity, the electrical conductivity (EC) of the soil water system rises according to the content of soluble salts in the soil giving rise to more of ion pairs on dissociation as it happens in case of a dilution solution. Conductivity was calculated with salt (conductivity) bridge using Standard Potassium Chloride solution for calibration. 20g of soil is shaken intermittently with 40ml of distilled water in a 150ml Erlenmeyer flask for one hour and allowed to stand. The conductivity of the supernatant liquid is determined with the help of the salt (conductivity) bridge. The measurement of EC is to be adjusted for a known temperature (usually 25° C) of the solution by setting the provided knob.

#### Total Organic Carbon

Total Organic Carbon was estimated by Walkley and Black (1934), rapid titration method. The organic carbon in the soil gets oxidized by chromic acid ( $1\text{N K}_2\text{Cr}_2\text{O}_7 + \text{conc. H}_2\text{SO}_4$ ) utilizing the heat of dilution of sulphuric acid. The unreacted dichromate is determined by back titration with 0.5N ferrous ammonium sulphate ( $(\text{NH}_4)_2\text{SO}_4\text{Fe}$ ) (redox titration). A combination of ortho Phosphoric Acid ( $\text{H}_3\text{PO}_4$ ) and Sodium Fluoride (NaF) in the reaction mixture give a sharper end point. Diphenylamine is used an indicator.

$$\text{Organic Carbon (\%)} = \frac{10 (B - T)}{B} \times 0.003 \times \frac{100}{\text{Wt. of soil}}$$

Where, *B* = Volume (in ml) of ferrous ammonium sulphate solution required for blank titration

*T* = Volume of ferrous ammonium sulphate needed for soil sample

#### Available Phosphorus ( $\text{P}_2\text{O}_5 \text{ kg ha}^{-1}$ )

Phosphorus in soil occurs as orthophosphate in several forms and concentrations and only a small fraction of the total amount present may be available to plants which are of direct relevance in assessing the phosphorus fertility level. A wide variety of methods are available for detecting available P in soil, we used Olsens's method in our study (Olsen and Sommers, 1982), 0.5M of  $\text{NaHCO}_3$  (pH 8.5 adjusted with 10% NaOH) is used as an Olsen's reagent. Soil samples were treated with activated carbon followed by

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Olsen’s reagent. A blank without soil was maintained simultaneously. Filtrates were colorimetrically analyzed for the available Phosphorus. An AR grade Potassium dihydrogen orthophosphate (KH<sub>2</sub>PO<sub>4</sub>) served as standard.

$$\text{Available P (kg/ha)} = R \times \frac{\text{Total volume of the extractant}}{\text{Volume of aliquot}} \times \frac{1}{\text{weight of soil taken}}$$

$$X \frac{2.24 \times 10^6}{10^6}$$

Where, R = µg P in the aliquot (from the standard curve)

$$\text{Olsen’s P (kg/ha)} = R \times \frac{50}{5} \times \frac{1}{2.5} \times 2.24 = \mu\text{g of P} \times 8.96$$

*Available Potassium*

This term refers to both exchangeable and water soluble forms of the nutrient present in the soil. These both are determined in the neutral normal ammonium acetate extract of soil. The ammonium ions provide a sharp and rapid separation from exchange complex while other cations bring about a gradual replacement of either lesser or greater amount of K which increases with the period of contact. The estimation of potassium in the extract was carried out with the help of flame photometer. Potassium chloride solution was used as a standard.

Available K (kg/ha)

$$= R \times \frac{\text{Volume of the extract}}{\text{Weight of the soil taken}} \times \frac{2.24 \times 10^{-6}}{10^{-6}}$$

Where, R= ppm of K in the extract (from the standard curve)

= ppm of K X 11.2

*Available Zinc, Iron, Copper & Manganese*

The quick estimation of soil micronutrient elements has become possible with the help of **AAS- Atomic Absorption Spectrophotometer**. Their deficiency as well as excessive occurrence affects the quality of the soil. In our study we dealt with the availability of four micronutrient elements viz Zn, Fe, Cu & Mn in the soils from hydrocarbon contaminated areas.

**DTPA (Diethylene-triamine penta acetic acid) reagent** was used to obtain extractable elements using **AAS**. (Respective standards were used) (Lindsay and Norvell, 1978).

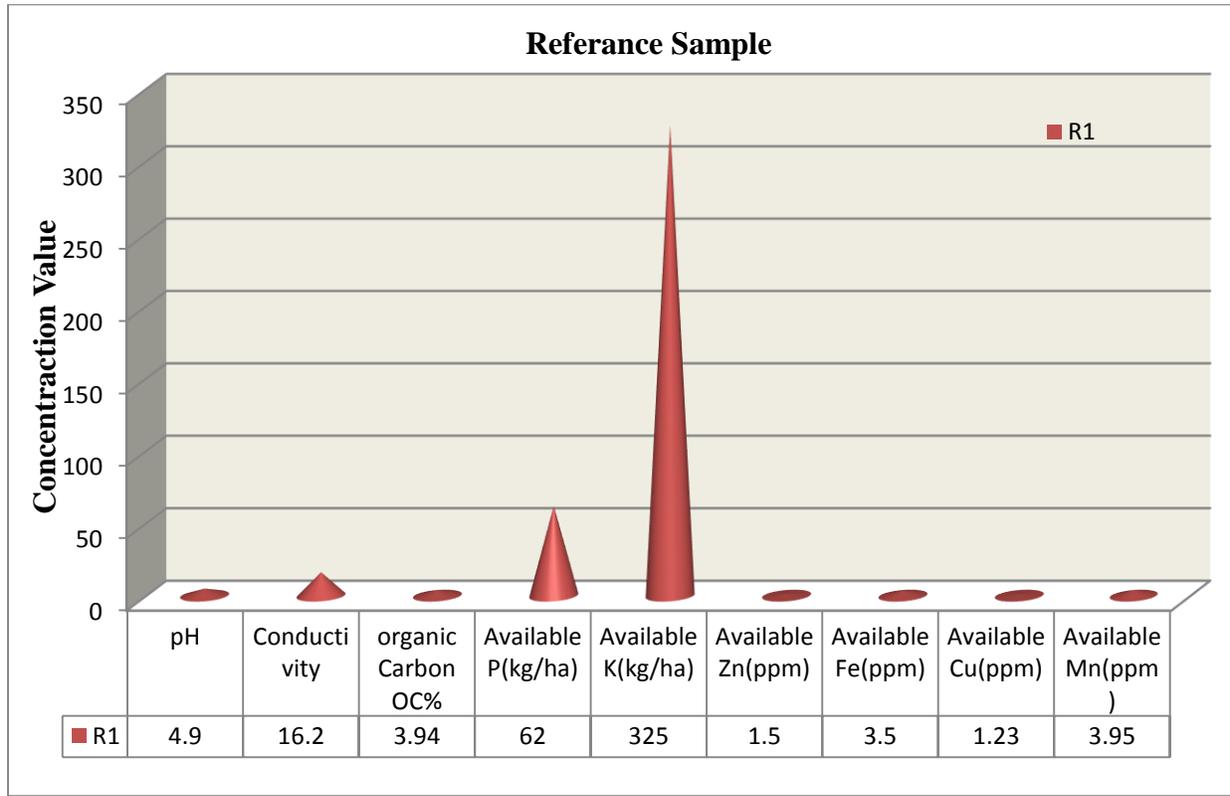
**RESULTS AND DISCUSSION**

The physicochemical studies of this work on soils of the PAH sites was designed to investigate the depletion in the quality of soil at the sites and suitability of the soil for land utilization, after over a decade of PAH.

Physicochemical analysis of the reference samples indicated a moderately good quality of soil. Alkaline pH and conductivity values were oblivion for the desert soils. Moderate values of organic carbon, available P & K and limiting values of heavy metals implicated no pollution (Figure 1).

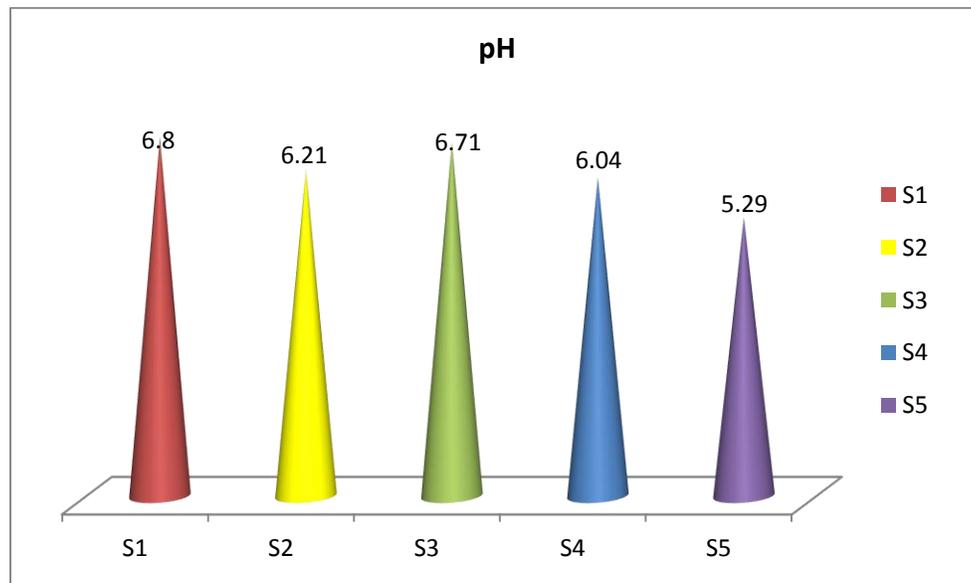
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*Reference Samples*



**Figure 1: The reference samples indicated a moderately good quality of soil**

*pH*



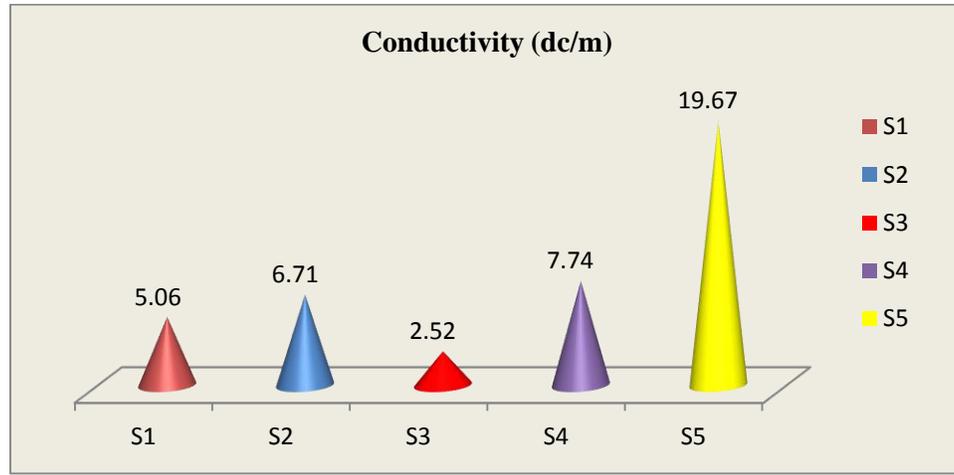
**Figure 2: The pH value of the soil lied within the acidic**

PAHs deteriorate the quality of soil making it more acidic. The pH values of the soil samples lied within the acidic range which may not support the growth of most crops that thrive on alkaline soil, leading to

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the loss of macro minerals needed for plant growth. Thus acidification of soil depletes important nutrient elements such as potassium, calcium and magnesium (Figure 2).

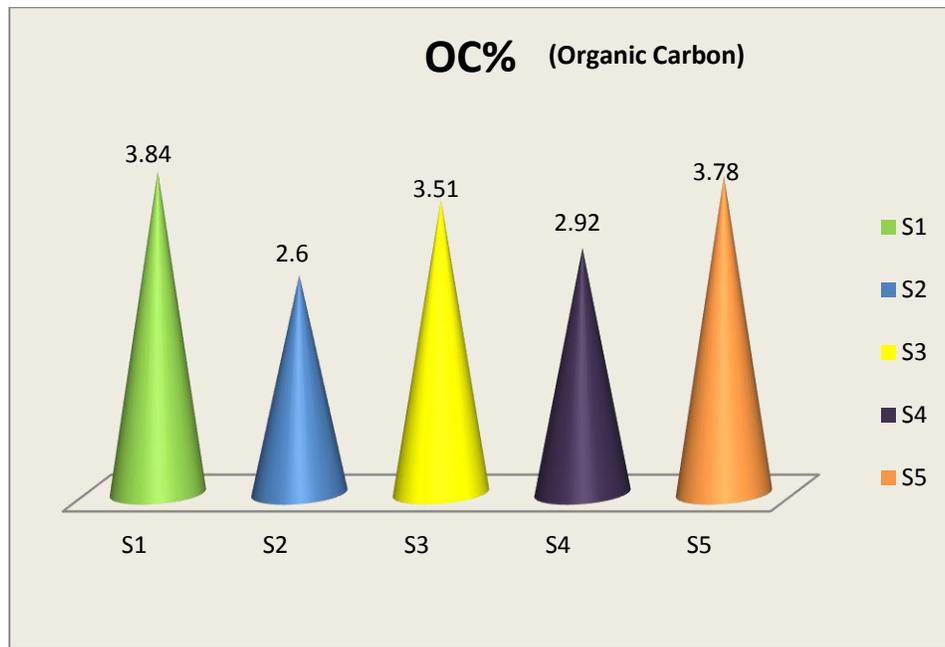
*Conductivity*



**Figure 3: The soil sample implicates to the high conductivity**

The High salt concentration in the soil sample implicates to the high conductivity Nwaogu and Onyeze (2010) (Figure 3).

*Organic Carbon*

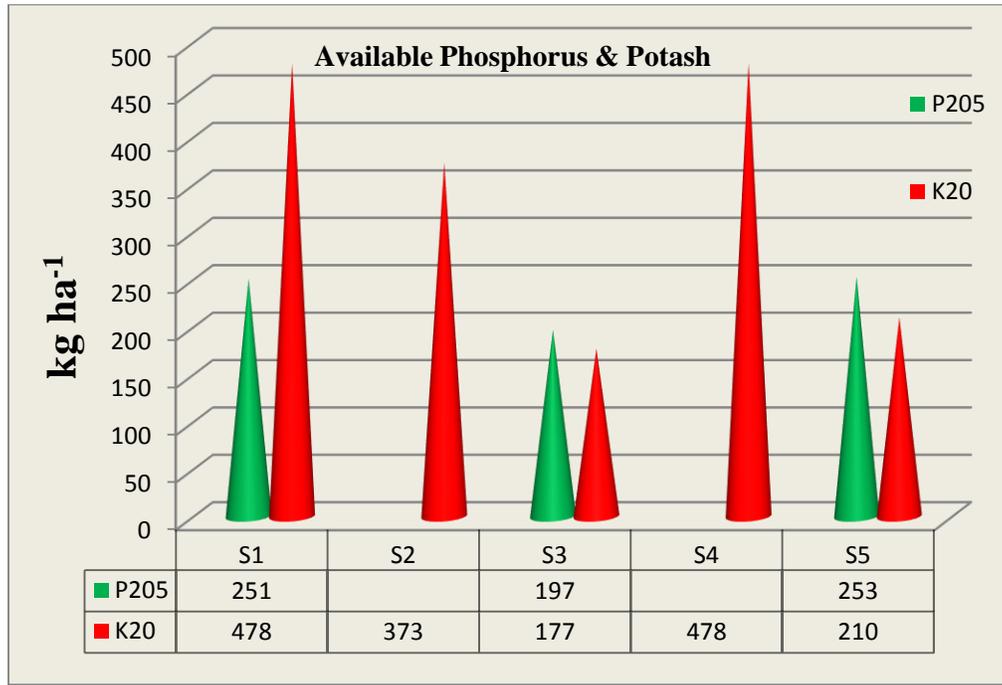


**Figure 4: A high percentage of organic carbon**

A high percentage of organic carbon in comparison to the reference samples (Figure 4) (Onyeagba and Isu, 2006). They have attributed this to the metabolic processes following PAHs that causes agronomical addition of organic carbon from petroleum hydrocarbon by reducing the carbon mineralizing capacity of the microflora. PAHs pollution overloads the soil with excess carbon leading to increased microbial population which tends to deplete the soil of nutrients (Osuji and Onojak, 2006).

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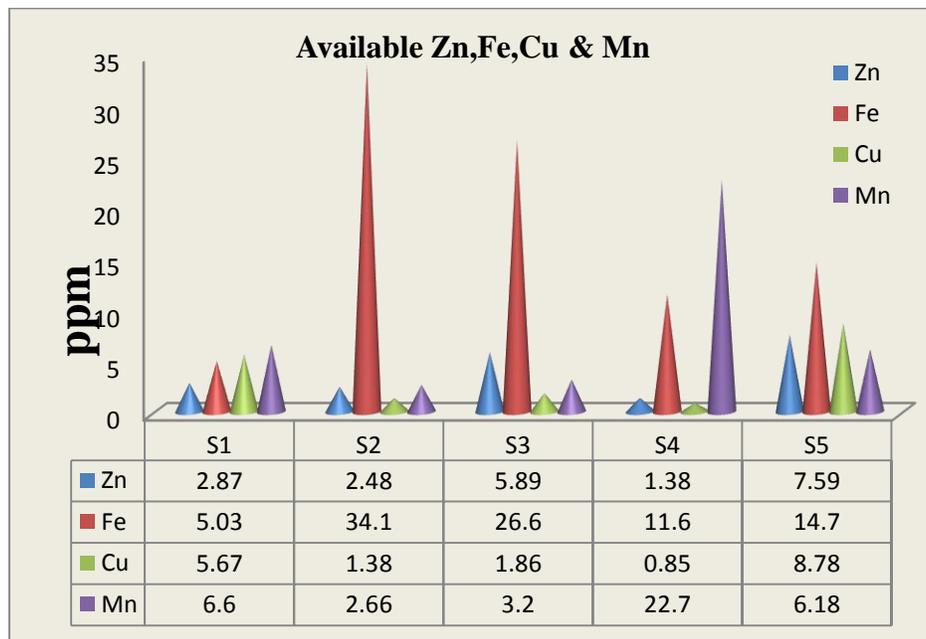
*Available Phosphorus & Potash*



**Figure 5: The levels of available Phosphorus and Potassium**

Figure: 5 shows Analysis of PAHs contaminated soils revealed remarkable fluctuations in levels of available Phosphorus and Potassium. Increase in oil spillage caused gradual depletion in the levels of these metals. Most of the PAHs contaminated samples lacked considerable amounts of available P and low levels of Potassium as compared to the reference samples.

*Available Zinc, Iron, Copper & Manganese*



**Figure 6: The concentration of zinc, iron, copper, and manganese**

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In Figure 6 shows Atomic Absorption Spectroscopic (AAS) analysis of the samples revealed increased concentration of zinc, iron, copper, and manganese as compared with the reference samples. The increase of these heavy metals in the polluted soil samples may be attributed to PAHs pollution in the sampling sites (Akubugwo *et al.*, 2009).

In view of our work, 5 soil samples were collected from hydrocarbon contaminated areas of Western Rajasthan to be analyzed physicochemical. The physicochemical properties were evaluated using different analytical methods. Physicochemical analysis of the reference samples indicated a moderately good quality of soil. Alkaline pH and conductivity values were oblivion for the desert soils. Moderate values of organic carbon, available P & K and limiting values of heavy metals implicated no pollution (figure 1).

The pH values of the soil samples lied within the acidic range which may not support the growth of most crops that thrive on alkaline soil, leading to the loss of macro minerals needed for plant growth. High salt concentration in the soil sample implicates to the high conductivity. The acidity of the polluted area causes a shift in normal metabolism of living things within an ecosystem (Figure: 3) Organic carbon increased with increase in pollution while, available potassium and phosphorus decreased with increase in acidification of the soil. High salt concentrations have lead to the high conductivity. When increase in oil spillage caused gradual depletion in the levels of these metals, Most of the PAHs contaminated samples lacked considerable amounts of available P and low levels of Potassium as compared to the reference samples. Increased concentration of zinc, iron, copper, and manganese as compared with the reference samples.

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### **REFERENCES**

- Akubugwo EI, Chinyere GC, Ogbuji GC and Ugbuagu EA (2009).** Physiochemical property of Enzyme activity in a refined oil contaminated soil in Isuikwuato L.G.A., Abia State. *Nigerian Society for Environmental Biology* **2** 79-84.
- Amellal N, Portal JM and Berthelin J (2001).** Effect of soil structure on bioavailability of polycyclic aromatic hydrocarbons within aggregates of a contaminated soil. *Applied Geochemistry* **16** 611-19.
- Bahuguna A, Lily MK, Mujal A, Singh RN and Dangwal K (2011).** A study on the physico chemical analysis of automobile contaminated soil of Uttarakhand, India. *International Journal of Environmental Science* (2) 380-388.
- Bates RG (1954).** *Electronic pH Determinations* (John Wiley and Sons Inc.) New York.
- Hussein ALN (2012).** Biodegradation of Crude Oil by Fungi Isolated from Gulf of Mexico. *Journal of Bioremediation and Biodegradation* **3**(4) 1-6.
- Kisku GC, Barma SC and Bhargava SK (2000).** Contamination of soil and plants with potentially toxic elements irrigated with mixed industrial effluent and its impact on the environment. *Water, Air, and Soil Pollution* **120** 121–37.
- Lily MK, Bahuguna A, Dangwal K and Garg V (2009).** Degradation of Benzo[a] Pyrene by a novel strain *Bacillus subtilis* BMT4i(MTCC 9447). *Brazilian Journal of Microbiology* **40**(4) 884–892.
- Lindsay WL and Norvell WA (1978).** Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* **42** 421-428.
- Minai Tehrani D and Herfatmanesh A (2007).** Biodegradation of aliphatic and Aromatic Fraction of Heavy crude Oil contaminated soil, A pilot Study. *Bioremediation Journal* **11**(20) 71 – 76.
- Nwaogu LA and Onyeze GOC (2010).** Environmental impact of Gas Flaring on Ebocha-Egbema, Niger-Delta. *Nigeria Journal of Biochemistry and Molecular Biology* **25**(1) 25-30.
- Olsen SR and Sommers LE (1982).** *Phosphorus Methods of Soil Analysis*, Part 2, 2<sup>nd</sup> edition. Agronomy Monogr 9, ASA and SSSA, Madison, WI 403-430.

**Research Article**

**Onyeagba RA and Isu NR (2006).** *General Microbiology*, 2<sup>nd</sup> edition (Research and Academic publishers) Imo State, Nigeria.

**Osuji LC and Onojake CM (2006).** Field Reconnaissance and Estimation of petroleum hydrocarbon and heavy metal content of soil affected by Ebocha oil spillage in Niger Delta, Nigeria. *Journal of Environmental Management* **79** 133-139.

**Sharma RK, Agrawal M and Marshall FM (2006).** Heavy metals contamination in vegetables grown in waste water irrigated areas of Varanasi, India. *Bulletin of Environmental Contamination and Toxicology* **77** 311–18.

**Smita C, Jyoti L, Vandana S and Chetan S (2012).** Assessment of Diesel Degrading Potential of Fungal Isolates from Sludge Contaminated Soil of Petroleum Refinery, Haryana. *Research Journal of Microbiology* **7**(3) 182-190.

**Walkley A and Black IA (1934).** An examination of the Degtjareff method for determining organic carbon in soils Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science* **63** 251-263.