J. Phytol. Res. **30** (1) : 69-81, 2017 **ISSN 0970-5767**

IMPACT OF PETROLEUM HYDROCARBONS ON PHYSICO-CHEMICAL PROPERTIES AND BACTERIAL POPULATION IN CONTAMINATED SOILS

ABHISHEK VASHISHTHA[1]*, GAUTAM KUMAR MEGHWANSHI[1], MADAN LOWRY[2] and D.P. JAROLI[2]
[1]Department of Microbiology, MGS University, Bikaner, Rajasthan, India.
[2]Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India.
* Corresponding author : E-mail: abhiv24@gmail.com

The petroleum hydrocarbons are potent source of pollution. Their large scale use, transport and refining has uplifted the concentration of these pollutants in the atmosphere. They are toxic compounds which are potentially carcinogenic and mutagenic. Presence of oily hydrocarbons in the soil influences the soil characters. In this study physico-chemical properties and microbial counts of three hydrocarbon polluted sites were compared with their uncontaminated counterparts. It was observed that the presence of oily hydrocarbons have a strong negative impact on the soil porosity, pH and soluble carbonates while a significant negative correlation exists between TPH and water holding capacity. The TPH significantly affected the organic matter, C:N ratio, and the bacterial population inhabiting in the contaminated soil.

Keywords: Bulk density; Carbonates; C:N ratio; Hydrocarbons; Pollution; Porosity; Water Holding Capacity.

Introduction

The human interventions, especially from last few decades have influenced a number of ecosystems. As a consequence of progress of civilian progress, diversified range of pollutants have accumulated in alarming proportions in the environment. The accumulation of xenobiotics in soil and water over the years has resulted in generation of thousands of hazardous waste sites. Historically, unintentional and deliberate spills of crude oil have been (and continue to be) a major source of pollution[1]. For example, approx. 6×10^7 barrels of oil was spread over 2×10^7 m^3 soil and 320 oil lakes were created across the desert during the first Gulf War in Kuwait[2]. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year[3].The organic pollutants entering the environment, chiefly through oil spills and incomplete combustion of fossil fuels, contaminate both groundwater[4] and agricultural lands[5]. Nitroaromatic compounds (NACs), polycyclic aromatics hydrocarbons (PAH's), NSO (nitrogen, sulfur and oxygen) compounds, other constituents of crude oil, wastes released from automobile garages, creosote discharges and many others together constitute a large and diverse group of chemicals responsible for producing widespread environmental pollution. The lighter and often toxic hydrocarbon components tend to volatilize into the atmosphere thereby reducing air quality and threatening the human and animal health. High concentration of sulfur compounds are also emitted in petrochemical waste streams that require urgent treatment. Some higher petroleum hydrocarbon compounds (Polyaromatic Hydrocarbons) show long term persistence (because of their stability and hydrophobicity) in the environment with half-lives extending over many years[6,7]. Longer persistence results in the bioaccumulation of these hydrocarbon pollutants. Hence, the elimination of pollutants and wastes from the environment has become an absolute requirement to promote the sustainable development of our society[8].

Petroleum hydrocarbons due to the following characteristics[9]- 1) chronic health effects (carcinogenicity/mutagenicity), (2) microbial recalcitrance, (3) high bio-accumulation potential, and (4) low removal efficiencies of traditional treatment processes, have been included in the list of priority pollutant across the globe[10].

Sites contaminated by such compounds need urgent remedial solutions[11,12], the search for which has revealed a diverse range of bacteria that can utilize the petroleum hydrocarbons as substrates. Upon long term exposure to hydrocarbons, any habitat can develop a bacterial population capable of mineralizing the pollutants. Such organisms often mineralize these pollutants by converting them into harmless products and in the process helps to clean up the environment[12]. Microorganisms comprise the most diverse forms of life and thus in fact represent the richest repertoire of molecular and chemical diversity in nature[13]. Now a day's research is increasingly being focused on the biological methods of degradation i.e. biodegradation and elimination of these pollutants. The timely bioremediation of the contaminated soils may also require some innovative practices[8].

The abiotic factors have an overall effect on the physiological activities of the microbes and hence on the bioremediation. The process of bioremediation depends upon many abiotic factors like type of hydrocarbons-chemical and physical status, site (nutrients, soil type, aeration, pH, cation exchange capacity etc), temperature, oxygen availability, salinity, carbon: nitrogen: phosphorus ratio and many more. The success of any remediation effort is dependent upon the environmental circumstances, such as local geology, mineralogy, soil condition - nutrient levels, pH, texture and structure along with temperature and precipitation.

The hydrocarbon contamination may influence the soil properties[14,15]. Petroleum compounds convert the local soil environment into anaerobic one[16], and thus they may alter the biological properties of the soil. Petroleum compounds can also influence the physico chemical properties of the soil like pH, organic content and others[17,18,19]. In the present study an attempt was made to find out the impact of high concentration of hydrocarbons on soil properties. We studied three hydrocarbon polluted sites. The physico-chemical properties and microbial counts of these soils were compared with the uncontaminated Rhizospheric and non-Rhizospheric soils which served as control.

Material and Methods

Sampling:

The southern outskirt of Jaipur city is densely occupied with a number of industries. The Sitapura Industrial area (Jaipur south) is known to harbor large number creosotes, garages, oil depots and many industries which liberate hydrocarbons as wastes bi-products. Soil samples were collected from the surface of the polluted soil of three motor garages located in Sitapura (26° 4878' N latitude, 75° 2034' E longitude) namely plot A (PA), plot B (PB) and plot C (PC). Samples were air dried for 96 hours at room temperature. After drying, they were spreaded over the spirit cleaned surface. The stone material, plant litter etc were removed by hands. Samples were passed through an 18 mesh size sieve to remove the other unwanted materials. In a similar manner uncontaminated rhizospheric soil (RZ) and non rhizospheric(N) were collected from the vicinity and brought to the laboratory. Both of these samples were used as a control.

Physico-Chemical analysis:

The bulk density and thus porosity of the soil was determined gravimetrically. Approximately 100 grams of soil was dug and the gap so formed was filled up with known volume of sand. The sample soil was dried in an oven at 100°C for 24 hours and then weighed by using a weighing balance. The dry weight per unit volume of soil gives the bulk density. The water holding capacity was determined by flooding the soil sample with water. After the excessive water was drained off, soil was dried at 100ºC temperature. Calculations based upon the weight of the dried soil and amount of water evaporated gives the water holding capacity of the soil[20]. Various physico-chemical factors like available carbonate and bicarbonate, pH, electric conductivity, $CaCO_3$and phosphorus were determined as per the standard titrimetric methods[20]. The micro Kjeldahl method as described elsewhere was used for the determination of nitrogen content[21]**.** The organic carbon and organic matter were determined as per the procedure described by Walkley and Black[22]**.** The phosphate, potassium, zinc, magnesium, iron and copper contents were analyzed by the Rajasthan Agriculture Research Institute, Durgapura, Jaipur.

Total Hydrocarbons Petroleum hydrocarbons (TPH) were quantified with the help of column chromatography and gas Chromatography as described elsewhere[23].

Biological Activities:

For soil respiration 100g of each soil sample was mixed with water equal to 33% of its maximum water holding capacity. A 15 ml test tube filled with N/10 NaOH solution was stringed up in the flask filled with soil and its mouth was sealed. Flasks were incubated at room temperature. The CO_2 evolved can convert NaOH to Na_2CO_3. The soil respiration was determined by titrimetric quantification of the excess amount of residue NaOH per week for a period of one month. Calculations were done as described by Dubey and Maheshwari[24]. The total heterotrophic bacterial population (THBP) in the soil samples was determined by serial dilution method and Nutrient Agar medium. The petroleum degrading bacterial population was determined by serial dilution method and minimal salt agar and broth medium (2.2g - K_2HPO_4, 0.73g - KH_2PO_4, 1.0g - $(NH_4)_2SO_4$, 0.2g - $MgSO_4.7H_2O$, 15.0g - Agar). The statistical analysis was done through IBM SPSS.

Results and Discussion

Biodegradation kinetics is strongly controlled by the type and population of the microorganisms present in the soil. The rate of biodegradation in soil is altered by factors affecting the microbial growth and activity. The ability of soil to absorb nutrients and hold water depends on its physical and chemical properties, texture and clay content, permeability, water holding capacity, bulk density, organic matter and cation exchange capacity[25]. All of these are known to affect the growth of microorganisms. On the other hand, presence of oily hydrocarbons influences the soil properties[19]. In fact, the soil environment is so complex and its properties are so interrelated that one cannot consider them separately when speaking of their influence on soil biology.

Soil Physicochemical-Biological Analysis-

Total Petroleum Hydrocarbons (TPH):

The gas chromatogram analysis of soil indicated that all the three contaminated samples were heavily contaminated with the petroleum contaminants. Soil sample II (PB) had the maximum hydrocarbons among all the contaminated samples (Table 2). This was expected as large quantities of petroleum compounds enter these garage soils.

Soil Bulk density and Porosity:

Bulk density and porosity are important physical properties which govern moisture content and air spaces in the soil. Soil sample II (PB) had the highest bulk density and lowest porosity, while rhizospheric soil had the lowest bulk density and highest porosity (Table 1). Bulk density of all the contaminated soil samples was higher than the un-contaminated samples; however the reverse was true for the soil porosity.

Table 1. Bulk Density and porosity of different samples

S. No.	Soil Sample	Bulk Density (g/cm^2)	Porosity (%)
1	Rhizospheric soil	1.26	52.45
2	Non- Rhizospheric soil	1.39	47.54
3	Sample I (PA)	1.89	28.68
4	Sample II (PB)	2.01	24.15
5	Sample III (PC)	1.90	28.30

As is evident from Table 5, there is strong correlation (0.862) between the total petroleum hydrocarbons and bulk density (and negative correlation with porosity).Crusting at the surface and compaction reduces the porosity and prevents entry of water and air into the soil, probably increasing surface runoff and erosion. The contaminated soils have very high content of hydrocarbons which binds up with the soil particles. As a result of this binding, the soil particles become dense and heavy. This cause fitting of the fine-textured soil particles into the macro pores so formed, thereby decreasing the soil porosity and enhancing its bulk density[26]. Low porosity indicates the persistence of an oxygen tense environment in the soil.

Soil pH and conductivity:

Contaminated soil samples were more acidic than their un-contaminated counterparts (Table 2). A significant negative correlation was observed (P0.05) between the concentration of petroleum hydrocarbons and pH (Table 6). Soil pH has significant correlation (P0.05) with the available

Table 2. Physico-chemical characteristics of the Soil Samples.Here: conductivity is in MOhms/cm; soluble carbonates, bi carbonates, chlorides are in– mg/100g; TPH is in– (µg\g of soil), $CaCO_3$, WHC, organic carbon, organic matter and total nitrogen are in– %, ND: Not detected. A-Non Rhizospheric Soil, B Rhizospheric, Cl^- Chloride Content.

Soil Sample	**Soil pH**	**Conductivity**	**Carbonates**	**Bi Carbonates**	**$CaCO_3$**	**Cl^-**	**WHC**	**Organic matter**	**Organic Carbon**	**Total N**	**C/N ratio**	**TPH**
A	8.3 ± 0.08	0.59± 0.007	19.5± 0.43	213.5 ± 6.36	2.5 ± 0.13	15.6± 0.50	25.6± 0.62	2.18±0.020	1.26 ± 0.012	0.14± 0.004	9.04± 0.31	41 ± 1.63
B	8.8 ± 0.05	0.69± 0.021	26.3± 0.653	170.8± 5.28	2.1 ± 0.082	18.4± 0.098	26.4± 0.65	3.13± 0.023	1.82 ± 0.014	0.19± 0.007	9.48± 0.28	53 ± 1.64
PA	7.9 ± 0.18	1.19± 0.037	ND	372.1 ± 5.86	3.0 ± 0.018	56.3± 1.01	18.0± 0.37	5.44± 0.077	3.16 ± 0.043	0.12± 0.003	26.32± 0.58	3,148 ± 64.76
PB	7.2 ± 0.07	0.78± 0.014	ND	383.4 ± 6.32	7.5 ± 0.17	63.0± 0.85	10.34± 0.66	6.65± 0.066	3.85 ± 0.037	0.11± 0.0029	29.82± 0.49	6,388 ±66.13
PC	7.6 ± 0.12	1.20± 0.05	ND	506.0 ± 6.81	4.0 ± 0.071	49.0± 0.37	19.57± 0.38	3.61± 0.062	2.1 ± 0.036	0.062 ± 0.0012	33.69± 0.85	2,085 ± 40.2
Soil Sample	Soil pH	Conductivity	Carbonates	Bi Carbonates	$CaCO_3$	Cl^-	WHC	Organic matter	Organic Carbon	Total N	C/N ratio	TPH

Table 3. Micronutrients in the soil samples

S. No.	**Soil Sample**	**Phosphate (Kg/hectare)**	**Potassium (Kg/hectare)**	**Zn (PPM)**	**Mg (PPM)**	**Iron (PPM)**	**Copper (PPM)**
1	Non Rhizospheric	36 ± 0.828	300 ± 5.71	2.79 ± 0.071	2.74 ±0.072	4.52 ± 0.073	0.38 ± 0.02
2	Rhizospheric	40 ± 0.86	280 ± 5.71	3.48 ± 0.16	3.12 ± 0.074	5.22 ± 0.059	0.32 ± 0.16
3	Sample I	30 ± 0.98	200 ± 7.25	9.00 ± 0.08	10.24 ± 0.074	32.0 ± 0.064	1.84 ± 0.056
4	Sample II	34 ± 0.71	290 ± 8.28	8.26 ± 0.14	12.20 ± 0.035	24.6 ± 0.45	1.74 ± 0.057
5	Sample III	36 ± 0.66	260 ± 4.89	10.32 ± 0.16	14.24 ± 0.075	29.00 ± 0.31	1.62 ± 0.020

soluble carbonates. The soil biology is significantly influenced by the ionic balance and pH acts as an indicator of this balance. Hydrocarbons are more persistent in acidic soil (pH <6) as compared to basic soil (pH >7)[27]. Hydrocarbons have greater half-life in acidic soil as compared to the basic ones. Pantelelis[27] obtained a negative correlation ($P<0.01$) between soil pH and hydrocarbon half-life. Mukred *et al,*[28] reported significant growth of hydrocarbon degrading bacterial isolates between pH 6.5 to 7.5. Microbial degradation of hydrocarbons often leads to the production of organic acids and other metabolic products. This is another reason for the low pH of the contaminated soil[29]. Sepahi*et al,*[29] reported a decrease of pH in the flasks inoculated with the hydrocarbon degrading bacteria. Soil pH is also influenced by the presence of microbial enzymes.

Table 4. Microbial activities of the soil samples

S. No.	**Soil Sample**	**THBP**	**PDBP**	**CO_2 Evolved (mg)**	**Carbon Mineralized (mg)**
1	Non Rhizospheric	$3.87 \pm 0.146 \times 10^{7}$	$8.71 \pm 0.81\times 10^{3}$	62.6 ± 2.23	17.07 ± 0.608
2	Rhizospheric	$4.97 \pm 0.913 \times 10^{8}$	$4.37 \pm 0.63 \times 10^{3}$	70.5 ± 2.00	19.22 ± 0.54
3	Sample I	$9.76 \pm 1.29 \times 10^{5}$	$4.03 \pm 0.812 \times 10^{4}$	11.4 ± 0.50	3.10 ± 0.12
4	Sample II	$6.63 \pm 1.17 \times 10^{5}$	$5.87 \pm 0.119 \times 10^{4}$	10.8 ± 0.31	2.94 ± 0.081
5	Sample III	$3.71 \pm 0.32 \times 10^{5}$	$4.0 \pm 0272 \times 10^{4}$	9.6 ± 1.51	2.61 ± 0.38

Table 5. Correlation analysis between Bulk density, Porosity and TPH

		Bulk Density	**Porosity**	**TPH**
Bulk Density	Pearson Correlation	1	-1.000**	.862
	Sig. (2-tailed)		.000	.060
	N	5	5	5
Porosity	Pearson Correlation	-1.000**	1	-.862
	Sig. (2-tailed)	.000		.060
	N	5	5	5
TPH	Pearson Correlation	.862	-.862	1
	Sig. (2-tailed)	.060	.060	
	N	5	5	5

**. Correlation is significant at the 0.01 level (2-tailed).

Soil sample III (PC) has the maximum conductivity among all soil samples. Normally electrical conductivity of the soil is <1 but here we found two contaminated samples having conductivity >1. This can be credited to the presence of high concentration of the charged particles in the contaminated soils. In the present study, a moderate positive correlation exists between the soil conductivity and TPH (Table 6). Depending on the charge, size and concentration in the soil, charged particles gets sorbed on the colloid surface or exchanged with other ions and released in the aqueous soil. The soil's ability to sorb and exchange ions is its 'exchange capacity' (both cation and anion exchange). Higher electrical conductivity also indicates high salinity in the contaminated samples[30].

Soil Carbonates and Bi Carbonates:

Presence and concentration of soil available carbonates influences the soil properties in many ways. Repeated experiments demonstrated total absence of soluble carbonates in all the contaminated soils samples (Table 2). The present study reveals a strong negative correlation between the concentration of the total petroleum hydrocarbons and soluble carbonates at 0.05 P (Table 6). One of the probable reasons for the depletion could be the microbial consumption of the available carbonates as a source of carbon and energy. They are an easy accessible source of carbon as compared to hydrocarbons hence it is expected that they would be used preferably by the microorganisms inhabiting in the contaminated sites. Another reason couldbe the downward leaching of the soluble carbonates in the lower horizon[31]. Because of the obvious reasons, the contaminated sites had very high content of bi-carbonates with sample III registering the maximum (Table 2).

Present results indicate that contaminated sites were more calcareous (more $CaCO_3$) than the uncontaminated ones. Additionally, high $CaCO_3$ content produces a cementing effect resulting in the formation of surface crusts. Such crusts results in restricted aeration and depletion of overall water holding capacity[32].A significant correlation exists between the $CaCO_3$ concentration and TPH (Table 6).

Soil Chloride Content:

Contaminated soil samples had 2-3 times more chloride as compared to the un-contaminated ones (Table 2). A significant correlation (P0.05) exists between the TPH and chloride (Table 6) while presence of chloride has a significant relation with pH. Presence of high chloride concentration in the contaminated sites highlights the presence of high salinity and electrical conductivity in these sites.

Soil Water Holding Capacity (WHC):

Maximum WHC was recorded in the rhizospheric soil. WHC of all the contaminated sites was found significantly lower than their un-contaminated counterparts (Table 2). WHC had a significant negative correlation with TPH (P0.01) (Table 6). The presence of contaminants like hydrocarbons, soil particle size, soil porosity etc directly influences the water holding capacity of the soil. The oily hydrocarbons form a coat over the soil particles. High bulk density (and thus compactness) interferes with the free movement of air and retention of water molecules across the soil pores. As a consequence the contaminated soils have low water holding capacity. WHC also depends upon the particle size of the soil and the presence of macro and micropores[32].

Organic Matter - Organic carbon, Nitrogen Content C/N ratio and Micronutrients:

Table 6. Correlation analysis between various Physico-chemical parameters

		pH	Conductivity	Carbonates	Bi Carbonates	Cl^-	WHC	Organic Carbon	N	C:N	$CaCO_3$	TPH
pH	Pearson Correlation	1	-.446	$.908^{*}$	-.828	$-.884^{*}$	$.924^{*}$	-.738	.816	$-.880^{*}$	-.873	$-.882^{*}$
	Sig. (2-tailed)		.452	.033	.083	.047	.025	.154	.092	.049	.053	.048
	N	5	5	5	5	5	5	5	5	5	5	5
Conductivity	Pearson Correlation	-.446	1	-.759	.819	.666	-.352	.357	-.691	.785	.058	.287
	Sig. (2-tailed)	.452		.137	.090	.220	.561	.556	.196	.116	.926	.639
	N	5	5	5	5	5	5	5	5	5	5	5
Carbonates	Pearson Correlation	$.908^{*}$	-.759	1	$-.921^{*}$	$-.947^{*}$	.836	-.731	.853	$-.955^{*}$	-.638	-.783
	Sig. (2-tailed)	.033	.137		.026	.014	.078	.160	.066	.012	.246	.117
	N	5	5	5	5	5	5	5	5	5	5	5
Bi Carbonates	Pearson Correlation	-.828	.819	$-.921^{*}$	1	.814	-.660	.491	$-.952^{*}$	$.970^{**}$	.523	.584
	Sig. (2-tailed)	.083	.090	.026		.093	.226	.401	.012	.006	.366	.302
	N	5	5	5	5	5	5	5	5	5	5	5
Cl^-	Pearson Correlation	$-.884^{*}$	.666	$-.947^{*}$	.814	1	$-.930^{*}$	$.901^{*}$	-.674	$.922^{*}$	.743	$.906^{*}$
	Sig. (2-tailed)	.047	.220	.014	.093		.022	.037	.212	.026	.151	.034
	N	5	5	5	5	5	5	5	5	5	5	5
WHC	Pearson Correlation	$.924^{*}$	-.352	.836	-.660	$-.930^{*}$	1	$-.934^{*}$	.562	-.798	$-.924^{*}$	$-.995^{**}$
	Sig. (2-tailed)	.025	.561	.078	.226	.022		.020	.324	.105	.025	.000
	N	5	5	5	5	5	5	5	5	5	5	5
Organic Carbon	Pearson Correlation	-.738	.357	-.731	.491	$.901^{*}$	$-.934^{*}$	1	-.313	.679	.787	$.954^{*}$
	Sig. (2-tailed)	.154	.556	.160	.401	.037	.020		.608	.207	.114	.012
	N	5	5	5	5	5	5	5	5	5	5	5
N	Pearson Correlation	.816	-.691	.853	$-.952^{*}$	-.674	.562	-.313	1	-.871	-.479	-.474
	Sig. (2-tailed)	.092	.196	.066	.012	.212	.324	.608		.055	.415	.420
	N	5	5	5	5	5	5	5	5	5	5	5
Ratio	Pearson Correlation	$-.880^{*}$	.785	$-.955^{*}$	$.970^{**}$	$.922^{*}$	-.798	.679	-.871	1	.652	.741
	Sig. (2-tailed)	.049	.116	.012	.006	.026	.105	.207	.055		.233	.152
	N	5	5	5	5	5	5	5	5	5	5	5
$CaCO_3$	Pearson Correlation	-.873	.058	-.638	.523	.743	$-.924^{*}$	.787	-.479	.652	1	$.925^{*}$
	Sig. (2-tailed)	.053	.926	.246	.366	.151	.025	.114	.415	.233		.024
	N	5	5	5	5	5	5	5	5	5	5	5
TPH	Pearson Correlation	$-.882^{*}$	.287	-.783	.584	$.906^{*}$	$-.995^{**}$	$.954^{*}$	-.474	.741	$.925^{*}$	1
	Sig. (2-tailed)	.048	.639	.117	.302	.034	.000	.012	.420	.152	.024	
	N	5	5	5	5	5	5	5	5	5	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Hydrocarbon contaminated sites receive continuous inputs of carbon and hydrogen. Continuous carbon input has enhanced the level of organic carbon and organic matter in the affected sites. In the present study contaminated sites were found to have very high concentration of hydrocarbons with sample II having the maximum (Table 2). Here we report a significant correlation between the organic content of the soil and TPH at 95% confidence level. Previous studies suggest a strong positive correlation between K_f (adsorption coefficient) of the pollutants and organic matter was found[27,33,34]. Nitrogen concentration in the contaminated soils was very low as compared to the uncontaminated ones (Table 2) while the C/N ratio in the contaminated soils was very high (about 3 times) as compared to uncontaminated soil samples. A strong positive correlation exists between the C:N ratio and the concentration of TPH was found (Table 6). The increased level of carbon disturbs the C:N and C:P ratios in the contaminated soils. These ratios are very crucial for the growth and development of soil microbial community[17,18]. The low levels of nitrogen and other micronutrients limit the microbial growth and thus severely affect the soil biodegradation potential. The very high C: N ratio inhibits many bacterial processes and negatively alters their stress management capacity. Low concentration of nitrogen limits the nucleic acid and protein synthesis and hence can also decline the rate of replication.

Table 7. Correlation analysis between THBP, PDBP, Soil respiration, TPH

		THBP	**PDBP**	**Soil respiration**	**TPH**
THBP	Pearson Correlation	1	-.681	.735	-.533
	Sig. (2-tailed)		.206	.157	.355
	N	5	5	5	5
PDBP	Pearson Correlation	-.681	1	-.943*	.944*
	Sig. (2-tailed)	.206		.016	.016
	N	5	5	5	5
Soil respiration	Pearson Correlation	.735	-.943*	1	-.789
	Sig. (2-tailed)	.157	.016		.113
	N	5	5	5	5
TPH	Pearson Correlation	-.533	.944*	-.789	1
	Sig. (2-tailed)	.355	.016	.113	
	N	5	5	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

Presence and concentration of micronutrients in any soil influences its microbial structure. Phosphate, Potassium, Zinc, Magnesium, Iron and Copper concentration was estimated in all the five soil samples. Zinc, Magnesium and Iron were present in very high concentration (2-5 times more) in all the contaminated sites while the concentration of other micronutrients was same in all the samples (Table 3).

Microbial Status and Activities:

The microbial status of the soil samples was observed by enumerating the total heterotrophic counts and the petroleum degrading population. These results were counterchecked by measuring the amount of CO_2 evolved and carbon mineralized (soil respiration) per unit weight of soil (Table 4).

Rhizospheric soil hosts the maximum number of total heterotrophic bacteria among all the soil samples while contaminated sample III (PC) had the minimum. On the contrary the petroleum degrading bacterial population was comparatively very high in the contaminated soil samples. These results were further confirmed by trapping and measuring the CO_2 evolved (soil respiration) from thesesoil samples. Here, a significant correlation exists between the PDBP and TPH at 95% confidence level. Similarly a strong negative correlation was observed between the TPH and Soil respiration (Table 7)

The low biological activity can be credited to low aeration (low-porosity), high C:N ratio, absence of soil available carbonates and presence of excess of hydrocarbons. In the conditions of stress (here presence of excess hydrocarbons), a bacterial cell require extra nutrients. Total absence or reduced levels of nutrients inhibits many bacterial species to proliferate. Presence of excess of hydrocarbons may also result in membrane toxicity. Enumeration of total viable bacterial counts and petroleum degrading bacteria are good indicators of the process of bacterial biodegradation potential. The growth dynamics of petroleum degrading bacterial population reflects the ability of inhabitant bacteria to degrade and utilize oil as source of carbon and energy[29,35]. To some extent, this explains the positive correlation of PDBP with TPH. In addition soil respiration can also be used to examine the process of mineralization of petroleum hydrocarbons[36].

Conclusion

The results of the present study have revealed a strong impact of oily hydrocarbons on the physico-chemical properties of the soil. The water holding capacity, porosity, soluble carbonates and bacterial population are negatively correlated with the concentration of the hydrocarbons. This impairs the biodegradation potential of the contaminated soil.

References

1. Trindade PVO, Sobral LG, Rizzo ACL, Leite SGF and Soriano AU2005, Bioremediation of a weathered and recently oil-contaminated soil fromBrazil: a comparison study. *Chemosphere*, **58** 515-522.
2. AL-Saleh ES and Obuekwe C 2005, Inhibition of hydrocarbon bioremediation by lead in a crude oil-contaminated soil. *Intl. Biodeter. Biodegrad*, **56** 1–7.
3. Kvenvolden KA andCooper CK 2003, Natural seepage of crude oil into the marine environment, *Geo-Marine Letters*, **23** (3-4) 140–146.
4. Schwarzenbach RP and J Westall 1981, Transport of nonpolar organic compounds from surface water to groundwater- Laboratory sorption studies. *Environ. Sci. Technol,* **15** 1360-1367.
5. Jones KC, Stratford JA, Tidridge P, Waterhouse KS and Johnston AE 1989, Polynuclear aromatic hydrocarbons in an agricultural soil: long-term changes in profile distribution. *Environ. Poll,* **56** 337-351.
6. Eriksson M, Sodersten E, Yu Z and Mohn WW 2003, Degradation of polycyclic aromatic hydrocarbons at low temperature under aerobic and

nitrate-reducing conditions in enrichment cultures from northern soils. *Appl. Environ. Microbiol*, **69** 275-284.

7. Kanaly RA and Harayama S2000,Biodegradation of high molecular weight polycyclic aromatic hydrocarbons by bacteria. *J. Bacteriol*, **182** 2059–2067.
8. Jain RK, Kapur M, Labana S, Lal B, Sarma PM, Bhattacharya D and Thakur IS 2005, Microbial diversity, Application of microorganisms for the biodegradation of xenobiotics. *Curr. Sci*, **89**(1) 101-112.
9. Herbes SE, Southworth GR and Gehra CW 1976, Organic Contamination in Aqueous Coal Conversion Effluents: Environmental Consequences and Research Priorities. In: *Trace substances in the Environment: A Symposium* (Ed.) Hemphill DD. Univ. of Missouri, Columbia, MO.
10. Zivelytea V, Valtersb K and Vasareviciusa S 2017, Comparison between passive remediation and bioremediation for the hydrocarbons contaminated soil clean up. *Energy Procedia*, **128** 339–344.
11. McIntosh P, Schulthess CP,Kuzovkina YA and Guillard K 2017, Bioremediation and phytoremediation of total petroleum hydrocarbons (TPH) under various conditions. *Int. J. Phytoremediation*, **19** (8)755-764.
12. Galdames A, Mendoza A, Orueta M, García I.S. de Soto, Sánchez M, Virto I and Vilas JL 2017, Development of new remediation technologies for contaminated soils based on the application of zero-valent iron nanoparticles and bioremediation with compost. *Resource-Efficient Technologies*, **3**(2) 166-176.
13. Yang S,Wen Xi, Shi Y, Liebner S, Jin H and Perfumo A 2016, Hydrocarbon degraders establish at the costs of microbial richness, abundance and keystone taxa after crude oil contamination in permafrost environments.*Scientific Reports*, **6**:37473.
14. Joseph J and Hari G 2015, Investigation on the Effects of Hydrocarbon Spillage on Soil Properties. *International Journal of Engineering Research and Technology (IJERT)*, **4**(10).
15. Brakorenko NN and Korotchenko TV 2016, Impact of petroleum products on soil composition and physical-chemical properties. *IOP Conf. Ser.: Earth. Environ. Sci*, **33**(01).
16. Sutton NB, Maphosa F, Morillo JA, Abu Al-Soud W, Langenhoff AA, Grotenhuis T, Rijnaarts HH and Smidt H 2013, Impact of long-term diesel contamination on soil microbial community structure. *Appl. Environ. Microbiol*, **79**(2) 619–630.
17. Wang X, Feng J and Wang J 2009, Petroleum hydrocarbon contamination and impact on soil characteristics from oilfield in Momoge wetland. *Environmental Science*, **30**(8) 2394–2401.
18. Wang X, Feng J and Zhao JM 2010, Effects of crude oil residuals on soil chemical properties in oil sites, Momoge Wetland, China. *Environ. Monit. Assess*, **161**(1) 271–280.
19. Wang Y, Feng J, Lin Q, Lyu X, Wang X and Wang G 2013, Effects of crude oil contamination on soil physical and chemical properties in Momoge

Wetland of China. *Chinese Geogr Sci*, **23**(6) 708–715.

20. Pandey J and Sharma MS 2003, Analysis of soil. In: Environmental Science: Practical and Field Manual. YashPublishing House, Bikaner, pp37-53.
21. Subbiah BV and Asija GL 1956, A rapid procedure for the determination of available nitrogen in soil. *Curr. Sci*, **25** 259-260.
22. Walkley A and Black IA 1934, An examination of the degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci*, **34** 29-38.
23. Pathak H, Vashishtha A, Jain PK, Nagmani A, Jaroli DP and Jain P 2010, Physico - Chemical properties of Petroleum contaminated soil collected from coastal areas of *Mumbai. Asian J. Exp. Sci*, **24** (1) 175-178.
24. Dubey RC and DK Maheshwari2004, Practical Microbiology. S. Chand and Company Ltd, New Delhi.
25. Hornick SB1983, The Interaction of Soils with Waste Constituents. In: *Land Treatment of Hazardous Waste* (Ed.) Parr JF, Marsh PB, Kla JM. Noyes Data Corp., Park Ridge, NJ, 4-19.
26. Brady N and Weil R 2002, The Nature and Properties of Soils, 13th Edition. *Prentice Hall*. Upper Saddle River, New Jersey, pp 960.
27. Pantelelis I, Dimitrios GK, Urania MS and Nikolas T 2006, Influence of Soil Physicochemical and Biological Properties on the Degradation and Adsorption of the Nematicide Fosthiazate. *J. Agric. Food Chem*, **54** (18) 6783–6789.
28. Mukred AM, Hamid AA, Hamzah A and Yusoff WMW 2008, Growth enhancement of effective Microorganisms for bioremediation of crude oil contaminated waters. *Pak. J. Biol. Sci,* **11**(13) 1708-1712.
29. Sepahi AA, Golpasha ID, Emami M and Nakhoda AM 2008, Isolation and Characterization of Crude Oil Degrading Bacillus spp. *Iran. J. Environ. Health. Sci. Eng,* **5**(3) 149-154.
30. Ayotamuno MJ, Kogbara RB, Ogaji SOT and Probert SD 2006, Bioremediation of crude oil polluted agriculture soil at Port-Harcourt, Nigeria. *Appl. Ener,* **83**(11) 1249-1257.
31. Lindsay WL 1979. Chemical equilibria in soils. *John Wiley and Sons,* Inc. New York, pp 449.
32. Massoud F 1972, Some physical properties of highly calcareous soils and their related management practices. FAO/UNDP Regional Seminar on Reclamation and Management of Calcareous Soils. Cairo, Egypt. November 7-December 2, 1972.
33. Simon L, Spiteller M, Haisch A and Wallnofer PR 1992, Influence of soil properties on the degradation of the nematicidefenamiphos. *Soil Biol. Biochem,* **24** 769-773.
34. Weber JB, Wilkerson CG and Reinhardt CF 2004, Calculating pesticide sorption coefficients (Kd) using selected soil properties. *Chemosphere.***55** 157-166.
35. Emtiazi G and Shakarami H 2004, Utilization of petroleum hydrocarbons by Pseudomonas sp. and transformed *Escherichia coli.African J. Biotechnol*, **4**(2) 172-176.

36. Rivera YE and Dendooven L 2004, Dynamics of carbon, nitrogen and hydrocarbons in diesel contaminated soil amended with biosolids and maize. *Chemosphere*, **54**(3) 379-386.