

Alteration in Physico-chemical Properties and Microbiological Analysis of Soil from various Hydrocarbon Contaminated sites across Maharashtra

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ABSTRACT Twelve soil samples were collected from oil-contaminated sites from Maharashtra State viz., Ahmednagar (SA, SB, SC and SD), Aurangabad (SE, SF, SG and SH), Nasik (SI and SJ), Pune (SK), and Nagpur (SL). The soil samples were analysed for physico-chemical parameters and microbiological characteristics. The physical parameters like colour, texture, temperature, moisture content and water holding capacity; as well as chemical parameters like pH, electrical conductivity, calcium carbonate, organic carbon and soil nutrients viz., nitrogen, phosphorous and potassium were estimated. Microbiological analysis involved the estimation of colony forming units/gm of soil sample. The pH of the samples ranged between 7.1 (SJ) and 8.2 (SL), which indicated presence of CaCO_3 in the soil, electrical conductivity ranged between 0.12 (SL) and 1.24 mS cm^{-1} (SK), calcium carbonate (%) ranged between 0.75 (SK) and 12.00 (SF), and organic carbon (%) ranged between 0.61 (SL) and 1.62 (SE). Soil nutrients like available nitrogen ranged between 116 (SF) and 402 kg/hectare (SE), available phosphorous ranged between 5 (SF, SL) and 32 kg/hectare (SK) and available potassium ranged between 123 (SF, SH) and 1098 kg/hectare (SE). The % organic carbon which determines the organic matter in soil was high. It was found that the potassium content in all the soil samples was more as compared to other nutrients like nitrogen and phosphorous. The residual hydrocarbon content in soil samples was in the range between 83.60 (SL) and 197.75 (SH) mg/kg of soil. The cfu/gm of soil ranged between 3.2×10^5 (SH) and 2.4×10^7 (SI). Microbiological analysis of contaminated soil revealed that the contaminated soils contained reasonable populations of bacteria. The work suggests future investigation on PAH-degradation capacities of the bacterial isolates, individually as well as in consortia so as to understand the prospects of employing these indigenous bacteria in bioremediation of PAH-contaminated soils.

Key Words: soil, physico-chemical properties, hydrocarbon extraction, indigenous bacteria,

I. INTRODUCTION

Environment and development are two sides of a coin. Developmental activities such as industrial, agricultural, transportation, etc., cause degradation and drastic change in every component of the environment through pollution (Gupta, 2002). On global basis four major factors viz., deforestation, overgrazing, agriculture and industrial development have been identified to cause soil degradation (FAO, 1994). Toxic substances released from industrial and modern agricultural activities (use of pesticides, weedicides, etc) contaminate soil by killing all microorganisms and change the chemical properties of the soil. As a result, fertility of soil is lost (Gupta, 2002). Due to wide range of industrial and agricultural activities, a high number of chemical contaminants are released into the environment, causing a significant concern regarding potential toxicity, carcinogenicity and potential for bioaccumulation in living systems of various chemicals in soil (Singh *et al.*, 2009). Toxic inorganic and organic chemicals are major contributors to the environmental contamination and pose a major health risk to the human population. Prevention of future contamination from these compounds presents an immense challenge (Evans, 2004).

Hydrocarbons are currently the main source of the world's energy resources, due to the energy they produce when combusted. This also makes them the world's main source of pollution in the case of spills and waste products (Atlas, 1981). All the operations in the petroleum industries, such as exploration and production of oil, transportation, refining, refined product handling and oily waste management activities are potential sources of water, soil and air pollution. The increase in environmental contamination through infiltration of petroleum products, both in water and on land, has led to a progressive deterioration of environmental quality (Kuhad & Gupta, 2009). The most notorious class of hazardous compounds found in petrol, diesel, oil as well as in coal tar and its derivatives, are the Polycyclic Aromatic Hydrocarbons (PAHs). PAHs are the common industrial pollutants and are found as co-contaminants in the environment (Joner *et*

al., 2002). The pollution of soils as a result of anthropogenic activities has received substantial attention in the past few decades, as compared with the previous two centuries of industrial activities (BASOL, 2008). Sixteen PAHs have been included in the United States Environment Protection Agency's priority pollutant list (Bogan *et al.*, 2001).

Keeping this in view, the present study was conducted to estimate the physico-chemical parameters and the microbial population in the petroleum contaminated soils across Maharashtra.

II. MATERIAL AND METHODS

2.1 Collection of soil samples

Approximately 1-2 kg of soil samples were collected in sterile polybags from petroleum/ oil-contaminated sites across major cities in Maharashtra viz., Ahmednagar (SA, SB, SC and SD), Aurangabad (SE, SF, SG and SH), Nasik (SI and SJ), Pune (SK), and Nagpur (SL) for analysis of physico-chemical parameters as per Somwanshi *et al.*, 2012 and Maiti, 2003, and microbial population was ascertained by colony count. The soil samples were mixed well, air dried in shade, ground to powder and were screened through a 2 mm sieve to remove debris. The subsamples were duly labeled, stored at - 4°C for further analysis. Hydrocarbon extraction was done using 50 ml acetone as a solvent (twice) for each 10 gm of soil sample. The sample after addition of solvent was placed on an orbital shaker for 15 minutes and then ultrasonicated for 30 minutes. Both the extracts were mixed and after centrifugation the solvent was filtered and dried using rotary evaporator. The microbial analysis was conducted within 24-48 hours of sampling.



Image 1: Soil Samples



Image 2: Hydrocarbon Extracts of Contaminated Soil

2.2 Soil microbial analyses

Indigenous microorganisms were extracted from the contaminated soil sample by mixing 1gm of soil in 10 ml of sterile saline solution in 50 ml Erlenmeyer flask for 2 hours on a shaker at 120 rpm as per Kastner *et al.* (1998). The soil particles were allowed to sediment for 30 minutes, the supernatant was diluted from 10⁻¹ to 10⁻⁵ dilutions, and the dilutions were plated on sterile nutrient agar plates. The inoculated plates were incubated at 37°C for 24 hours. The colony forming units per gm (cfu/gm) of soil sample were determined using serial dilution agar plate method (Dubey & Maheshwari, 2012).

III. RESULT AND DISCUSSION

A range of environmental parameters those are physical, chemical or biological in nature affect bioremediation of petroleum contaminants (Huesemann, 1995; Mphekgo *et al.*, 2004). The first and foremost decisive factor for designing a bioremediation program is to study the indigenous microflora of the system and to analyse the physical properties and chemical composition of soil. Nutrient availability plays an important role in adaptation of microbes and their growth on hydrocarbons. Two major nutrients, nitrogen and phosphorous, are considered to be the most important, as they are required for incorporation of carbon into the biomass (Pritchard & Costa, 1991).

In this light, the physico-chemical characteristics like colour, texture, temperature, moisture content, water holding capacity, pH, electrical conductivity, calcium carbonate, organic carbon; soil nutrients like nitrogen, phosphorous and potassium were estimated according to Somwanshi *et al.* (2012) and Maiti (2003) (Table 1 & 2).

Table 1. Physical Parameters of Contaminated Soils						
Sr. No.	Soil Sample	Parameters				
		Colour	Texture	Temperature (°C)	Moisture Content (%)	Water Holding capacity (%)
1.	A	Brown	Silty clay	26	42	28.14
2.	B	Black	Sandy	32	52.6	64.0
3.	C	Brown	Sandy loam	28	50	35.42
4.	D	Black	Loam	30	59	39.12
5.	E	Brown	Sandy loam	27	40	28.36
6.	F	Black	Sandy	29	48	36.0
7.	G	Slightly brown	Silt	26	52	62.14
8.	H	Black	Clay loam	28	53.1	64.0
9.	I	Pale Brown	Sandy	25	46	31.52
10.	J	Brown	Sandy	31	42	26.0
11.	K	Black	Clay loam	30	57.4	66.0
12.	L	Brown	Sandy loam	25	52	38.32

Table 2. Chemical Parameters of Contaminated Soils										
Sr. No.	Soil Sample	Parameters						Soil Nutrients		
		pH	EC mS cm ⁻¹	Calcium carbonate (%)	Organic carbon (%)	Organic Matter (%)	N	P	K	
1.	SA	7.7	0.54	3.25	0.75	1.29	248	17	963	
2.	SB	7.4	1.18	7.62	0.63	1.08	365	12	874	
3.	SC	7.6	0.38	6.45	1.16	1.99	121	21	523	
4.	SD	7.7	0.49	3.46	0.67	1.15	213	13	832	
5.	SE	7.4	1.09	7.50	1.62	2.79	402	10	1098	
6.	SF	7.6	0.26	12.00	0.61	1.05	116	05	123	
7.	SG	8.0	1.11	11.50	1.02	1.75	172	25	806	
8.	SH	7.6	0.34	2.00	0.89	1.53	210	11	123	
9.	SJ	7.1	0.32	5.75	1.40	2.41	304	15	650	
10.	SK	7.6	1.24	0.75	1.05	1.81	119	32	762	
11.	SL	8.2	0.12	11.75	0.61	1.05	176	05	336	

In the present study, the physical parameter like soil colour was found to be black for soil samples SB, SD, SF, SH and SK; brown for samples SA, SC, SE, SJ and SL; whereas slightly brown for sample SG. The colour of the soil sample SI was distinguishingly pale brown. Diverse soil texture was observed during the study. The soil samples from sampling sites SB, SF, SI and SJ were sandy, from SC, SE and SL were sandy loam, from SD was laom, from SH and SK were clay loam; whereas, it was silty clay from SA. Temperature of the soil samples was found to be in the range of 25°C (SI) to 32°C (SB). Water is a regulator of physical, chemical and biological activities of soil (Maiti, 2003). The moisture content (%) of the soil samples was in the range of 40 (SE) to 59 (SD); whereas, the water holding capacity (%) of the soil samples was in the range of 26 (SJ)

to 66 (SK).

Of the various chemical properties, one of the enlightening attributes of soil is pH. Since biological activities are pH specific, determination of pH is very important (Somwanshi *et al.* 2012). In the present study, the pH of the soil samples was in the range of 7.1 (SJ) to 8.2 (SL) (Fig. 1). Maximum pH 8.2 of the soil from sampling site SL indicated availability of nutrients. It suggested neutral to slightly alkaline nature of the soil samples. Electrical Conductivity (EC) measures soil salinity and is indicative of the ability of an aqueous solution to carry an electrical current. The EC of the soil samples was in the range of 0.12 mS cm⁻¹ (SL) to 1.24 mS cm⁻¹ (SK) (Fig. 2). High EC (i.e. > 1 mS cm⁻¹) of soil from sampling site (SB, SE, SG and SK) indicated presence of higher soluble salts in the soil, which dissociates into their respective cations and anions and impart conductivity. Whereas, minimum EC of soil from sampling site (SL) suggested less soluble salts in the soil. Gupta (2000) has put forth that higher the concentration of ions in solution, more is its electrical conductance (less the resistance to electric current) and thus, the measurement of EC can be directly related to the soluble salt concentration.

In the present investigation, the calcium carbonate content (%) was in the range of 0.75 (SK) to 12.00 (SF) (Fig. 3). Organic carbon content of soil comprises of 48 to 58 % of total organic matter (Somwanshi *et al.*, 2012). Organic carbon (%) was found to be in the range of 0.61 (SL) to 1.62 (SE) (Fig. 4). Organic matter content influences many soil properties like capacity of soil to supply N, P, S and trace elements, infiltration and retention of water, degree of aggregation of overall soil structure, cation exchange capacity and soil colour (Maiti, 2003). In the present study, the organic matter (%) content was found to be in the range of 1.05 (SL) to 2.79 (SE) (Fig. 5).

The major part (>90%) of soil nitrogen exists as complex combination in the organic matter fraction and becomes available after breakdown to simple forms followed by mineralization (Gupta, 2000). The availability of N is associated with the activity of microorganisms, which decompose the organic matter and transforms N into its mineral form (Somwanshi *et al.*, 2012). In the present study, available nitrogen was in the range of 116 kg ha⁻¹ (SF) to 402 kg ha⁻¹ (SE) (Fig. 6), which indicated presence of microorganisms in soil that decompose the organic matter. Nitrogen was found to be in low concentration limit i.e. below 250 kg/ha in samples SA, SC, SD, SF, SG, SH, SK and SL; whereas, in sample SB, SE and SJ, it was found to be in medium concentration limit i.e. 250-500kg/ha.

Phosphorous in soil ranges from 0.01 to 0.3 % and occurs in several forms and combinations. The total amount of phosphorous present in soil is not in the available form; only a small fraction of it may be available, which is of direct relevance in assessing phosphorous levels (Gupta, 2000). Phosphorous is a key nutrient and plays a vital role in large number of enzymatic reactions, which depend upon phosphorylation (Mall *et al.*, 2004). In the present study, the phosphorous content was in the range of 05 kg ha⁻¹ (SL, SF) to 32 kg ha⁻¹ (SK) (Fig. 7). In samples SF and SL the phosphorous concentration was low i.e. below 10 kg/ha, in samples SA, SB, SD, SE, SH and SJ, phosphorous concentration was medium i.e. 10-20 kg/ha; whereas, it was high i.e. above 20 kg/ha, in samples SC, SG and SK.

Potash (K₂O) in Indian soil ranges from 0.05-3.5% out of which 95% part is present in complex form, 1-10% part in relatively non-available form, and 2% part is in available form. The term 'available potassium' includes both exchangeable and water soluble forms of potassium present in soil (Gupta, 2002). In the present study, the available potassium was in the range of 123 kg ha⁻¹ (SH) to 1098 kg ha⁻¹ (SE) (Fig. 8). In samples SF and SH, the concentration of available potassium was medium i.e. 100-250 kg/ha; whereas, it was high to very high i.e. above 250 kg/ha in samples SA, SB, SC, SD, SE, SG, SJ, SK and SL. The availability of N, P, K, Ca and Mg are maximum when pH ranged between 5.0 to 8.5 (Murali & Rao, 2005). Pollution of soil results in increased pH, organic carbon, available N, P, K; while reducing the water holding capacity (Kumar, 2002). This coincides with the present study.

Spatial variation in the residual hydrocarbons content was found among the contaminated soil samples collected from different sampling stations having a history of hydrocarbon contamination (Table 3). The highest residual hydrocarbon concentration 197.75 mg/kg was found in SH and the lowest concentration 83.60 mg/kg was found in SL (Fig. 9). PAHs from soil are absorbed by plants and crops and ultimately they enter into human body and cause carcinogenicity to human beings (Mcgowin *et al.*, 2001). Therefore, they require more attention to cleanup (Bishnoi *et al.*, 2009).

The fertility of soil depends not only on its chemical composition, but also on the qualitative and quantitative nature of microorganisms inhabiting it (Subba Rao, 2004). Therefore microbial analysis of the soil samples was carried out, which involved the estimation of bacterial load in the contaminated soil in the form of cfu/gm of soil samples (Table 4).

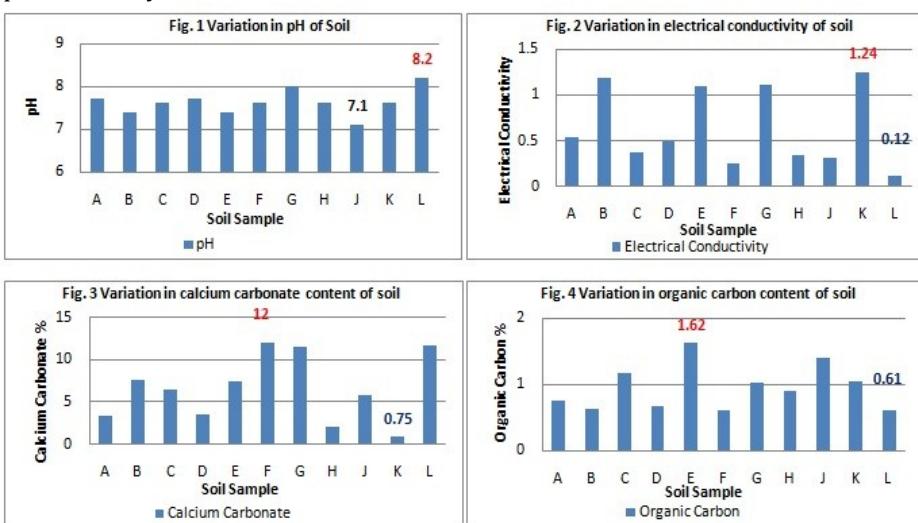
Table 3. Mean Concentrations of Residual Hydrocarbons (mg/kg) in Soils

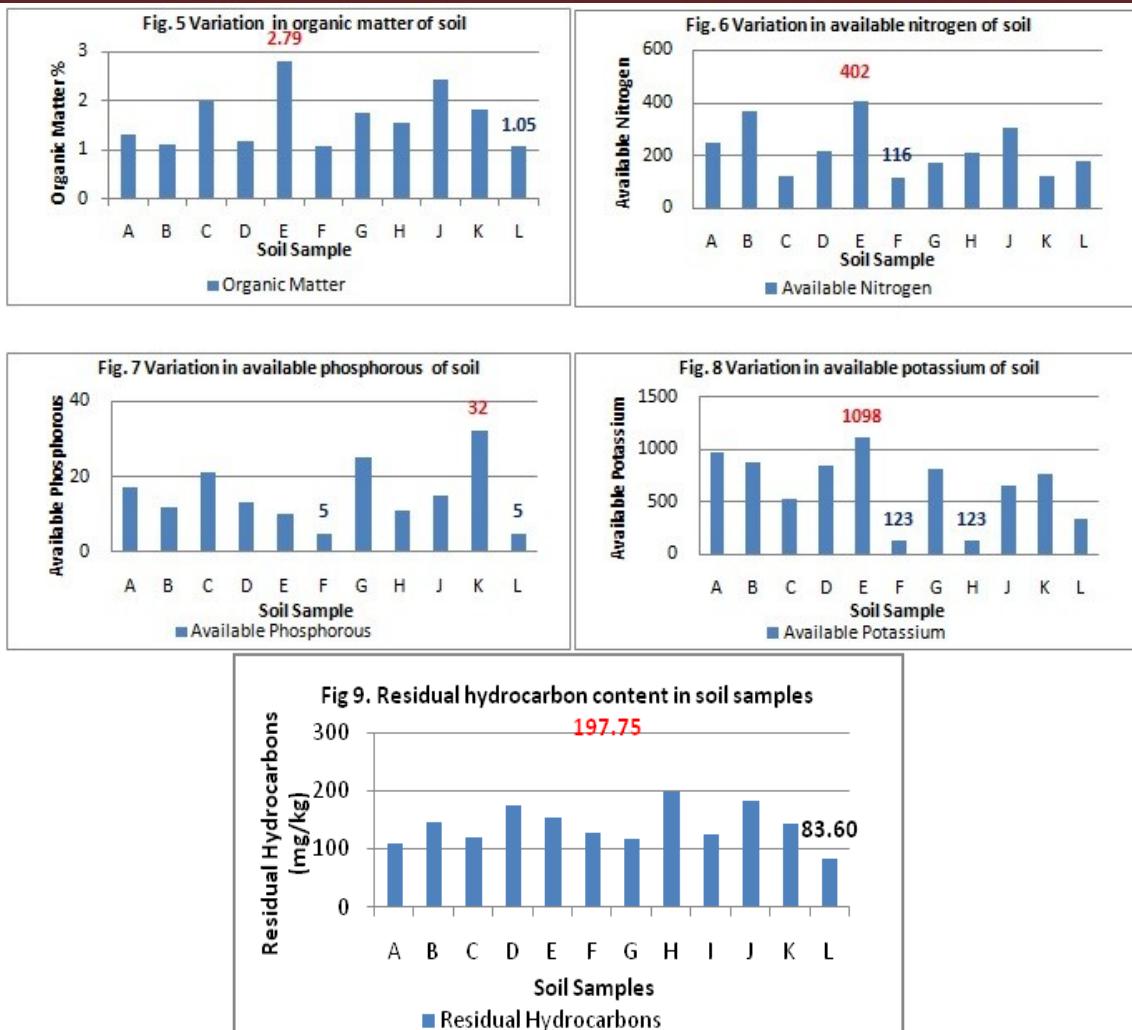
Sr. No.	Soil Sample	Residual Hydrocarbons
1	A	108.85
2	B	146.57
3	C	119.37
4	D	174.82
5	E	155.00
6	F	128.70
7	G	117.00
8	H	197.75
9	I	123.95
10	J	182.93
11	K	143.43
12	L	83.60

Table 4. Initial Soil Microbiology

Sr. No.	Soil Sample	CFU/gm of Soil
1	A	8.8×10^5
2	B	7.6×10^5
3	C	8.2×10^5
4	D	1.2×10^6
5	E	6.2×10^5
6	F	2.8×10^6
7	G	1.4×10^6
8	H	3.2×10^5
9	I	2.4×10^7
10	J	7.8×10^6
11	K	9.6×10^5
12	L	3.6×10^6

In the present study, the cfu/gm of soil samples was in the range of 3.2×10^5 (SI) and 2.4×10^7 (SH), which indicated the ability of the microorganisms to adapt and grow in contaminated soils. According to Van Hamme *et al.* (2003), a large number of microorganisms belonging to a variety of genera are able to utilize hydrocarbons as the sole source of carbon and energy, and these microorganisms are widely distributed in nature. Forsyth *et al.* (1995) demonstrated that significant bioremediation occurred when population of the hydrocarbon-degrading microorganism in the soil is more than 10^5 CFU/gm of soil. This coincides with the results of the present study.





IV. CONCLUSION

The soil parameters like EC point towards the presence of high amount of soluble salts in the soil samples. Available nitrogen in soil suggests the presence of soil microorganisms which decompose the organic matter and convert it into available form of nitrogen. Varying amounts of phosphorous (i.e. low, medium as well as high concentration) was observed in the soil samples. It is to be noted that available potassium was found to be in very high concentration in most of the samples, which influences the microbial population. Considerable amount of residual hydrocarbon in the collected samples indicated that they were contaminated by petroleum products. Contaminated soil microbiology (in the form of Colony Forming Units) showed that the contaminated soils contain reasonable populations of bacteria (3.2×10^5 - 2.4×10^7 cfu/gm of soil) which can be harnessed for biodegradation. Hence, it can be concluded that the bioremediation of PAH-contaminated soils in Maharashtra may be considered as a feasible option to reduce soil pollution resulting from petroleum products.

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