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Degradation of Petroleum Fractions in Soil Under Natural Environment: A Gravimetric and Gas Chromatographic Analysis

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1. Introduction

The modern civilization depends on exploration, distribution and use of petroleum and thousands of products derived from it. The environment all throughout the world has been very badly damaged due to these chemicals. While pollution of the atmosphere has been caused almost totally by burning of the petroleum products, the leaks, spillages and accidental fallout of petroleum products and the crude petroleum itself have greatly affected land and water resources. The oil field areas always receive a large amount of effluents rich in crude oil and land degradation is a common phenomenon. While using different fractions of petroleum (gasoline, kerosene, diesel etc.) spills and leakages cannot be avoided and in industrial areas, garages and other places, a large amount of it flows to the nearby areas. All types of oil have amazing spreading power and once a leak occurs, the oil may spread horizontally as well as vertically depending upon soil conditions, moisture, temperature etc. The lighter fraction being volatile are easily removed by evaporation and other physical processes, but the heavy components such as aromatics – simple and polycyclic, Hopanes etc. remain in the soil for a very long time unless biodegraded by soil micro organisms. The detection of presence of some carcinogenic hydrocarbons viz. PAH has made the situation a matter of serious concern. In most cases; the pollutant oil exerts its detrimental effects before it degrades into harmless and simple compounds. Hence a study on the type of degradation mechanism, the measures which can expedite the process of bioremediation and how the soil parameters are influenced by oil pollutants and their degradation is demanded by situation. The present investigation intends to gains some true knowledge on the matter based on experimental findings.

2. Soil is a depleting natural resource

Human race is dependent on a number of gifts of the nature. One such gift of nature is the Soil resource. It has been defined as the thin layer of the earth's crust in which biological activities take place. The beneficial activities of soil are multidimensional. Animal life is absolutely dependent on plant kingdom for food. Plants absorb nutrients from soil and convert it into a form acceptable to animal kingdom. Thus it is one of the best supporters of

life. Soil is made of weathered rock material and organic matter. Weathering, the physical and chemical breakdown of rocks is the first step in the soil forming process. The process of soil formation is a very long and slow geological process. The process is cyclic, because the rocks are changed to unconsolidated particles, which may be eventually be cemented together by other chemical and physical mechanisms to yield new rocks. The slowness of this soil producing process indicates that soil is a depleting natural resource when subjected to erosion, loss of fertility or pollution. Soil fertility, the ability of the soil to supply the plants with their essential nutrient elements, directly determines what crops can be grown on a soil and the nutritional value of these crops for man and animals.

3. Land degradation due to hydrocarbon pollutants

By virtue of the properties such as a portable, dense energy source and as the base of many industrial chemicals; Petroleum is one of the world's most important commodities. Today about 90% of fuel needs are met by oil. The presence of the oil industry has significant environmental impacts. Accidents and routine activities such as seismic exploration, drilling, and generation of polluting wastes affect the society. Oil extraction is often environmentally damaging. Leaks, spillages and accidental fallouts of petroleum and its various fractions are of frequent occurrence. Offshore exploration and extraction of oil disturbs the surrounding marine environment. Crude oil and refined fuel spills from tanker, underground pipelines, and ship accidents and have damaged soil resource, ecosystem in many parts of the world.

Land degradation due to this is a common phenomenon in the modern world. Since the early part of the twentieth century, the damaging effect of the hydrocarbons from oil has been known. Many workers reported the damaging effect of petroleum and its various fractions on soil and water resources (Young, 1935). It was reported that crude oil can sterilise soils and prevent crop growth for various period of time. The duration of the damaging effect depends largely on the degree and depth to which the soil is saturated with the oil. The damage that the oil does is due mostly to the prevention of the plant from obtaining sufficient moisture and from ramifying its roots very little is due to toxicity, as such (Plice, 1948).

Out of various processes of disappearance of oils from polluted soil, microbial degradation of pollutants is one of the most important ones (Davies & Westlaki, 1979). Crude petroleum and its fractions are converted into soil organic matter by bacteria and fungi. During the conversion, the organisms, which are free lives, fix fairly large amount of atmosphere nitrogen in the soil. Later this nitrogen becomes available for plant growth and the organic matter improves soil physical conditions. Based on general principle of hydrocarbon degradation, a programme of rehabilitation such as liming, fertiliser addition, and frequent tilling is considered to be broadly applicable for all types of mineral oils and experiments showed that these are really effective measures (Dibble & Bartha, 1979). Dry micro organisms contain approximately 14% N, 3% P and 1% S in the form of proteins, nucleic acids, polysaccharides and low molecular weight compounds. On the other hand, Petroleum products are composed of hydrogen and sulphur and essentially no phosphorous in their environment in order to grow on hydrocarbons as their carbon and energy sources (Rosenberg, 1993). This application of nutrients especially that of Phosphorous seems to be an effective means of rehabilitation and experiment showed that this is really so (Reynolds & Walworth, 2000). On the contrary, studies showed that the presence of Polychlorinated

Biphenyls negatively impact fossil fuel degradation (Hoeppel et.al, 1995).Out of several methods to expedite the process of degradation of Petroleum Hydrocarbons and to rehabilitate an oil inundated soil such as Bioremediation, Polyencapsulation, Vapour Transport, Land Farming, Alcohol Flooding etc. Bioremediation is considered effective and environmentally benign. Before taking up remediation measures emergency clean up operation is an important activity.

4. Methodology

Soil sample having no history of oil pollution were taken, polluted by known quantity of oil using emulsifier- some with nutrients, some with oxidising agents and some without any additive and the stated experiment was done by placing indoor in polyethene bags and maintaining the conditions necessary for microbial activities (Table-1). Series with B, C, D and E are similar to series A except that the oil is replaced by Kerosene oil, Diesel oil, Lubricating oil and Residual oil respectively. Gravimetric determination was done by withdrawing 20 g polluted soil sample from each bag and by recovering the oil by soxhlet extraction method using Petroleum ether as solvent after definite time interval. Gas Liquid Chromatography (GLC/GC) is the method of choice for rapidly and accurately analysing the volatile substances. It is also applicable to non volatile ones due to availability of higher column temperatures. Different compounds maintain similar sequence in the chromatogram analyzed under same system of column and identical conditions.

Sample No.	Mass of Crude Oil	Mass of Nutrient	Mass of Soil	Total mass	Concentration of Crude Oil	Volume of Emulsifier	Volume of H_2O_2 added
-	g	g	g	g	ppm	mL	mL
Set-1							
1A0	0	0	3000	3000	0	2	0
1A1	3	0	2997	3000	1000	2	0
1A2	15	0	2985	3000	5000	2	0
1A3	30	0	2970	3000	10000	2	0
1A4	45	0	2955	3000	15000	2	0
1A5	60	0	2940	3000	20000	2	0
Set-2							
2A0	0	10	2990	3000	0	2	0
2A1	3	10	2987	3000	1000	2	0
2A2	15	10	2975	3000	5000	2	0
2A3	30	10	2960	3000	10000	2	0
2A4	45	10	2945	3000	15000	2	0
2A5	60	10	2930	3000	20000	2	0
Set-3							
3A0	0	0	3000	3000	0	2	10
3A1	3	0	2997	3000	1000	2	10
3A2	15	0	2985	3000	5000	2	10
3A3	30	0	2970	3000	10000	2	10
3A4	45	0	2955	3000	15000	2	10
3A5	60	0	2940	3000	20000	2	10

Table 1. Sample Numbers and their Contents possessing Crude Oil.

5. Results and discussions

A few important parameters of the soil such as Texture, pH, Hydraulic Conductivity, Bulk Density, Water Holding Capacity, Soil Organic Carbon, NPK nutrients etc. were determined before the start, in between, and at the end of the experiment(Sing et al, 2000) (Table-2).

Parameter	Value	Unit	Parameter	Value	Unit
Texture	Silty clay	-	pH	6.86	-
Bulk Density	1.082	g cm^{-3}	WHC	41.80	%
Electrical Conductivity	0.43	$mScm^{-1}$	SOC	1.00	%
Hydraulic Conductivity	0.073	cm min^{-1}	SOM	1.724	%
Particle Density	2.650	g cm^{-3}	Porosity	59.17	%

Table 2. Values of Physico-Chemical Parameters of Soil.

Remains of hydrocarbons and their oxygenated derivatives in the above mentioned soil bags were extracted by soxhlet extraction using Petroleum Ether as solvent and gravimetric determination was done (Table-3). It was found that the NPK supplementation is an effective measure of rehabilitation of oil inundated soil. Supply of nutrients to the soil helps micro organisms to grow abundantly, consequently the degradation becomes faster. Hydrogen peroxide decomposes some of the organic matter present in the polluted soil and it also contributes towards the degradation to some extent. It has been found that more than 50% of the applied mass undergoes degradation in average in all the three sets of crude oil experiment during the first two months of placement. The activities of micro organisms are highly dependent on temperature. Ambient temperature was also recorded during the period of the experiment. The monthly average of the ambient temperature during the

Sample	Oil Added	Amount of Oil Recovered (g) after				
No.	g	1 month	2 month	3 month	4 month	5 month
1A1	3	01.975	01.650	01.111	00.142	00.030
1A2	15	12.797	09.375	07.979	05.634	03.832
1A3	30	17.715	14.200	13.339	10.842	06.327
1A4	45	34.567	20.051	19.018	17.878	10.001
1A5	60	41.400	30.645	23.243	19.835	10.770
2A1	3	01.321	01.200	00.915	00.098	00.022
2A2	15	11.850	08.476	07.700	04.545	02.875
2A3	30	16.715	13.100	11.990	08.895	05.500
2A4	45	31.922	18.025	14.019	13.950	07.290
2A5	60	37.950	27.409	20.890	14.160	09.110
3A1	3	01.613	01.400	00.965	00.110	00.000
3A2	15	12.040	08.734	07.800	05.312	03.005
3A3	30	16.973	13.362	12.319	09.312	05.780
3A4	45	33.727	19.632	16.737	14.499	08.560
3A5	60	38.419	29.319	21.783	15.322	09.234

Table 3. Balance Amount of Crude Oil Recovered against Months after subtracting the mass found in Controls.

period of the experiment is given (Table 4). The experiment was done during summer in the city of Guwahati, where the average of ambient temperature was 28.24 ± 0.40^0C in the range of 26.89-29.19^0C with negligible decreasing trend (correlation coefficient, r = -0.20). These conditions along with other physico-chemical parameters were favourable for microbial growth (Khan & Anjaneyulu, 2005).

Temp.	At 1st month	At 2nd	At 3rd	At 4th	At 5th	At 6th	Aver-age	Standard Error	Correlation Coefficient
^{0}C	27.28	29.19	28.65	28.81	28.63	26.89	28.24	±0.40	-0.20

Table 4. Average Ambient Temperature during the Period of Experiment.

Many agencies working on Hydrocarbon pollution use GC technique to estimate hydrocarbon pollution. G.C. analysis of the oil recovered from three samples viz. 1A5, 2A5 and 3A5 (Plate-1) from three sets after two months since placement have shown that number of components become 22, 37 and 39 respectively including solvent (Sarma et al, 2005a). Hydrogen Peroxide decomposes organic compounds from soil. Hence a good number of volatile components under GC conditions have been found in the 3A5 sample. Later these components escape from soil as carbon dioxide and a fraction fixes with soil. In the mass determination of the remaining hydrocarbons, it has been found that degradation in the second set i.e. set having applied NPK is highest. It means that it has already degraded and given away more compounds from the polluted soil sample. It has been seen from the chromatograms that there are no components with retention time in the range of 16.315 -43.247 minutes, 17.682 - 40.729 minutes and 11.589 - 42.677 minutes respectively in1A5, 2A5 and 3A5. A large number of components with minute differences in their retention time are seen in the chromatograms. Table- 5 represents some of such peaks between 1A5 and 2A5. The corresponding peaks in 2A5 appeared with an advance retention time of 0.452 minute in average belonging to the range of 0.571 to 0.392 minute. There are no corresponding peaks in 2A5 for five compounds present in 1A5 (Table-6). Probably these are the compounds degraded completely as a result of profuse microbial activities due to application of nutrients. Table (Table-7) represents the list of peaks which are found in 2A5 but not in 1A5. These are the peaks of compounds formed due to higher microbial activities as a result of application of nutrients. Number of such components are 20 and these compounds occupied a minute area % (average 0.63 %) of the chromatogram in the range

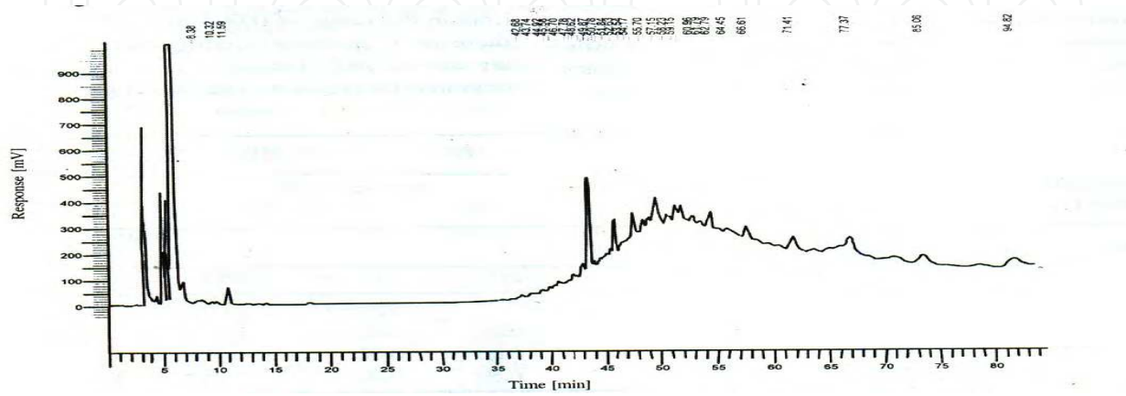

Plate 1. GC Chromatogram of oil recovered after 2 months of placement of sample 3A5.

Sl.	Serial Number	1A5		2A5		Minute
No.	As in 1A5 Chromatogram	Retention Time	Area % Report	Retention Time	Area % Report	Advance in Ret. time
1	6	12.271	19.38	11.700	45.15	0.571
2	7	12.810	1.27	12.363	3.54	0.447
3	8	13.288	0.39	12.839	1.92	0.449
4	9	14.538	6.36	14.080	12.32	0.458
5	10	15.286	2.11	14.835	6.17	0.451
6	11	15.823	0.12	15.339	2.17	0.484
7	12	16.315	3.09	15.860	5.38	0.455
8	13	43.247	0.68	42.845	0.30	0.402
9	14	48.772	1.85	48.380	0.26	0.392
10	15	49.805	2.47	49.363	0.78	0.442
11	16	50.389	23.34	49.993	5.26	0.396
12	17	51.724	0.08	51.275	0.16	0.449
13	18	52.736	0.27	52.320	1.03	0.416
14	19	53.152	4.70	52.744	1.55	0.408
15	20	55.236	2.48	54.832	0.83	0.404
16	21	56.304	1.06	55.814	0.46	0.490
17	22	59.828	0.21	59.261	0.10	0.567

Table 5. List of Similar Peaks in GC Analysis Report of 1A5 and 2A5.

Sl No in Chromatogram	1	2	3	4	5
Retention Time in minute	09.053	09.355	09.774	10.497	10.992
Area %	05.33	07.42	03.87	02.13	11.41

Table 6. List of peaks found in 1A5 giving no corresponding peak in 2A5.

Sl. No.	Sl. No. of peak	Retention Time in minute	Area %	Sl. No.	Sl. No. of peak	Retention Time in minute	Area %
1	4	13.220	0.30	11	25	53.630	0.07
2	6	14.513	2.56	12	28	56.367	0.34
3	10	17.682	0.65	13	29	57.210	2.31
4	11	40.729	0.09	14	30	58.320	0.16
5	12	42.359	0.04	15	32	59.782	0.25
6	14	44.121	0.73	16	33	61.080	0.04
7	15	45.072	0.07	17	34	62.848	1.74
8	16	45.714	0.16	18	35	66.697	0.61
9	17	46.270	0.15	19	36	71.440	1.52
10	18	46.840	0.66	20	37	77.554	0.18

Table 7. List of peaks found in 2A5 but not in 1A5.

of 0.04 to 2.56%. Table (Table-8) represents 14 peaks due to same compounds between 1A5 and 3A5 with their time of appearance. The corresponding peaks in 3A5 appeared after an average advancement of retention time of 0.589 minute in the range of 0.502 to 0.682 minute. These common components are the examples of some of the compounds which are resistant to degradation during this period. There are no corresponding peaks for eight compounds present in 1A5 (Table-9).These have already disappeared in 3A5. Probably these are the compounds degraded completely as a result of degradation activities due to application of

-	Sl. No.	1A5		3A5		
Sl No	as in 1A5 Chromatogram	Retention Time	Area % Report	Retention Time	Area% Report	Advance in Retention Time
1	1	9.053	5.33	8.383	0.68	0.670
2	2	9.355	7.42	8.726	0.52	0.629
3	5	10.992	11.41	10.325	0.46	0.667
4	6	12.271	19.38	11.589	3.68	0.682
5	13	43.247	0.68	42.677	0.22	0.570
6	14	48.772	1.85	48.249	1.15	0.523
7	15	49.805	2.47	49.228	2.97	0.577
8	16	50.389	23.34	49.868	20.99	0.521
9	17	51.724	0.08	51.132	1.19	0.592
10	18	52.736	0.27	52.229	1.50	0.507
11	19	53.152	4.70	52.630	7.23	0.522
12	20	55.236	2.48	54.734	4.93	0.502
13	21	56.304	1.06	55.695	3.11	0.609
14	22	59.828	0.21	59.154	5.48	0.674

Table 8. List of Similar Peaks in GC Analysis Report of 1A5 and 3A5.

Sl. No.	1	2	3	4	5	6	7	8
Peak No	3	4	7	8	9	10	11	12
RT in minute	9.774	10.497	12.810	13.288	14.538	15.286	15.823	16.315
Area %	3.87	2.13	1.27	0.39	6.36	2.11	0.12	3.09

Table 9. List of peaks found in 1A5 giving no corresponding peak in 3A5.

hydrogen peroxide. Table-10 represents the list of peaks which are found in 3A5 but not in 1A5. These are the peaks of compounds formed due to higher microbial activities that occurred on the compounds. Number of such components are 25 and these compounds occupied a minute area (average 1.84%) of the chromatogram in the range of 0.06 to15.19%.

GC analysis of the oil, recovered after 1 month and 2 months in the sample 2A3 gave a total of 17 and 24 peaks respectively. There is no peaks during12.217 to 47.277 minutes and during 15.303 to 44.055 minutes in the samples after 1 month and after 2 months respectively. Out of the 17 compounds obtained after I month, it appears that 14 compounds are still present in oil recovered after two months. It means that these components could resist degradation in the second month. These are placed in table (Table-11). These compounds appear 0.232 to 0.387 minute (average 0.275 minute) advance in the sample

obtained after 2 months. This change in retention time is probably due to minor change of experimental conditions during transit. The three components, which appeared at 12.217,

Sl. No.	Sl. No. of peak	RT in minute	Area %	Sl. No.	Sl. No. of peak	RT in minute	Area %
1	6	43.743	0.10	14	27	57.153	15.19
2	7	44.919	0.13	15	28	58.234	0.95
3	8	45.577	0.43	16	30	59.705	3.71
4	9	46.125	0.40	17	31	60.961	0.29
5	10	46.702	0.84	18	32	61.788	0.09
6	11	47.778	0.14	19	33	62.792	4.38
7	13	48.620	0.59	20	34	64.446	0.09
8	16	50.340	0.66	21	35	66.609	3.74
9	17	50.776	0.06	22	36	71.413	6.76
10	19	51.836	1.66	23	37	77.370	0.35
11	22	53.537	0.40	24	38	85.062	0.44
12	23	54.169	0.17	25	39	94.824	0.54
13	26	56.245	3.78	-			

Table 10. List of peaks found in 3A5 but not in 1A5.

-	Sl. No.	After 1 m		After 2 m		Minute
Sl No	1 month report	Retention Time	Area %	Retention Time	Area %	Advance in RT
1	1	10.941	0.90	10.686	9.58	0.255
2	3	47.277	0.71	47.001	2.21	0.276
3	4	49.850	0.44	49.571	3.13	0.279
4	5	50.406	9.43	50.159	11.01	0.247
5	6	51.771	0.27	51.465	0.25	0.306
6	7	52.736	7.70	52.496	7.66	0.240
7	8	53.171	3.43	52.928	4.94	0.243
8	9	55.244	1.65	55.012	2.77	0.232
9	10	56.314	4.17	56.037	3.29	0.277
10	11	56.893	0.55	56.594	0.29	0.299
11	12	57.621	18.27	57.365	17.62	0.256
12	13	58.132	3.12	57.860	2.74	0.272
13	14	59.848	0.81	59.561	4.05	0.287
14	15	63.516	20.07	63.129	13.55	0.387

Table 11. List of Similar Peaks in GC Analysis Report of 2A3 after 1Month and 2Months.

72.525 and 81.026 minutes with area percent report of 25.47, 1.85 and 1.17 respectively in the sample after 1 month got disappeared in the second month. It has also been seen that another 10 peaks due to 10 new compounds appear in the sample after 2 months (Table- 12). These compounds seem to be the degradation products of various hydrocarbons. These peaks have area percent in the range of 0.11 to 11.00 with an average of 1.69%.

In the GC analysis of the sample 3A3 after 1 month and after 2 months, it has been seen that there are 11 and 33 components respectively including that for the solvent. There are no peaks appear in between 20.082 to 46.835 and in between 11.662 to 42.728 minutes in the two

Sl. No.	Sl. No. of peak	Retention Time in minute	Area %	Sl. No.	Sl. No. of peak	Retention Time in minute	Area %
1	1	6.823	0.86	6	7	44.055	0.11
2	2	7.256	0.17	7	14	53.817	0.38
3	3	9.225	2.24	8	15	54.476	0.39
4	4	13.335	0.61	9	21	58.546	0.24
5	5	15.303	0.92	10	24	71.991	11.00

Table 12. List of peaks found in the sample 2A3 after 2 months but not in after 1 month.

samples respectively. As many as 8 numbers of peaks of components present after 1 month in 3A3 can be linked to same number of components present in the extract obtained after 2 months. Table (Table-13) represents these peaks. The corresponding peaks appear in the sample after 2 months with an advance retention time average of 0.06 minute in the range of 0.011 to 0.126 minute. Corresponding peaks for the rests three viz. at retention time 20.082, 62.788 and 71.406 minutes are not present in the second month. It implies that these components disappear during the month. The other 25 components which appear in the sample after 2 months are probably due to formation of derivatives as a result of degradation activities. These components have an average area percent of 1.42 in the range of 0.05 to 6.33 (Table-14).

-	Sl. No.	After 1 month		After 2 months		Minute
Sl No	1 m report	Retention .Time in minute	Area %	Ret. Time in minute	Area %	Advance in RT (minute)
1	1	11.732	36.21	11.662	2.83	0.070
2	3	46.835	1.00	46.709	0.31	0.126
3	4	49.952	2.45	49.912	25.59	0.040
4	5	52.303	5.93	52.275	1.28	0.028
5	6	52.734	1.43	52.681	7.05	0.053
6	7	54.822	0.89	54.777	4.20	0.045
7	8	55.863	3.19	55.760	4.85	0.103
8	9	57.207	16.13	57.196	18.27	0.011

Table 13. List of Similar Peaks in GC analysis report of 3A3 extracted after 1 and 2 months.

The 17 and 11 peaks found respectively in the GC chromatogram of samples 2A3 and 3A3 after 1 month of placement, possess 9 peaks (Table-15) having a very narrow range of 0.414 to 0.454 minute difference in the retention time except one which appeared at a difference of 0.728 minute. The average difference in retention time is 0.469 minute. It has been seen that larger number of components disappeared from 3A3 at the same time compared to 2A3. Out of 24 and 33 peaks respectively found in the GC chromatogram of samples 2A3 and 3A3 after 2 months 15 similar peaks have been identified and presented in table (Table- 16). Compared to number of peaks found in 2A3, the corresponding peaks in 3A3 appear in

advance by a narrow range of 0.169 to 0.365 minutes where the average value obtained is0.261 minute. The components responsible for these peaks are resistant to degradation at least up to two months under the experimental conditions.

Sl. No.	Sl. No. of peak	Retention Time (minute)	Area %	Sl. No.	Sl. No. of peak	Retention Time (minute)	Area %
1	1	8.258	0.10	14	17	51.891	2.70
2	2	8.807	0.07	15	20	53.620	0.71
3	4	42.728	0.33	16	21	54.246	0.05
4	5	44.020	0.48	17	24	56.266	3.55
5	6	44.956	0.26	18	26	58.276	1.02
6	7	45.629	0.65	19	27	59.196	6.33
7	8	46.177	0.62	20	28	59.742	2.19
8	10	47.826	0.21	21	29	61.125	1.71
9	11	48.299	1.18	22	30	62.843	1.70
10	12	48.682	0.86	23	31	66.631	1.29
11	13	49.288	5.85	24	32	71.433	0.26
12	15	50.393	1.20	25	33	77.479	0.49
13	16	51.195	1.81	-	-	-	-

Table 14. List of peaks found in the sample 3A3 after 2 months but not in after 1 month.

	Sl. No.	2A3-1M		3A3-1M		Minute
Sl. No.	2A3 report	Retention Time	Area %	Retention Time(min)	Area %	Advance in Retention time
1	2	12.217	25.47	11.772	36.21	0.440
2	3	47.277	0.71	46.835	1.00	0.442
3	5	50.406	9.43	49.952	2.45	0.454
4	7	52.736	7.70	52.303	5.93	0.433
5	8	53.171	3.43	52.734	1.43	0.437
6	9	55.244	1.65	54.822	0.89	0.422
7	10	56.314	0.417	55.863	3.19	0.451
8	12	57.621	18.27	57.207	16.13	0.414
9	15	63.516	20.07	62.788	21.04	0.728

Table 15. List of Similar Peaks in GC Analysis Report of 2A3and 3A3 after one month.

Gas Chromatographic analysis of recovered oil from kerosene oil polluted soil (B-set) after 2 months of placement showed that number of components at 1B5 and 3B5 (Plate-2) became 17 and 35 respectively. Gravimetric determination shows that mass of oil recovered in the samples having applied Hydrogen Peroxide is less, whereas number of components in the same is more than that of the sample without Hydrogen Peroxide (Table-17). It indicates that Hydrogen Peroxide removes the pollutants by degrading into smaller compounds. There are no peaks appeared in between the retention time ranges 4.838 to 32.566 minute in1B5 and in the range of 9.611 to 32.669 minute in 3B5. It appears that for every peak in the 1B5 sample, there is a corresponding peak in the 3B5 sample (Table-18) with an average delay of 0.115 minute in the range of 0.095 to 0.190 minute. All peaks present in 1B5 have

been found in 3B5. Excluding peak at serial number 9, all other similar peaks in the table have higher peak area at 1B5. Besides these, there are about 18 peaks in 3B5 but not in 1B5, out of which the peaks at 2.716 appears to be for the solvent. These 18 peaks (Table-19) seem to be for some degradation products.

.	Sl. No.	2A3	2A3	3A3	3A3	Minute
Sl. No	As in 2A3	Retention Time	Area % Report	Retention Time (minute)	Area % Report	Advance in Retention time
1	8	47.001	2.21	46.709	0.31	0.292
2	9	49.571	3.13	49.288	5.85	0.283
3	10	50.159	11.01	49.912	25.59	0.247
4	11	51.465	0.25	51.195	1.81	0.270
5	12	52.496	7.66	52.275	1.28	0.221
6	13	52.928	4.94	52.681	7.05	0.247
7	14	53.817	0.38	53.620	0.71	0.197
8	15	54.476	0.39	54.246	0.05	0.230
9	16	55.012	2.77	54.777	4.20	0.235
10	17	56.037	3.29	55.760	4.85	0.277
11	18	56.594	0.27	56.266	3.55	0.328
12	19	57.365	17.62	57.196	18.27	0.169
13	21	58.546	0.24	58.276	1.02	0.270
14	22	59.561	4.05	59.196	6.33	0.365
15	23	63.129	13.55	62.843	1.70	0.286

Table 16. List of Similar Peaks in GC chromatogram of 2A3 and 3A3 extracted after two months.

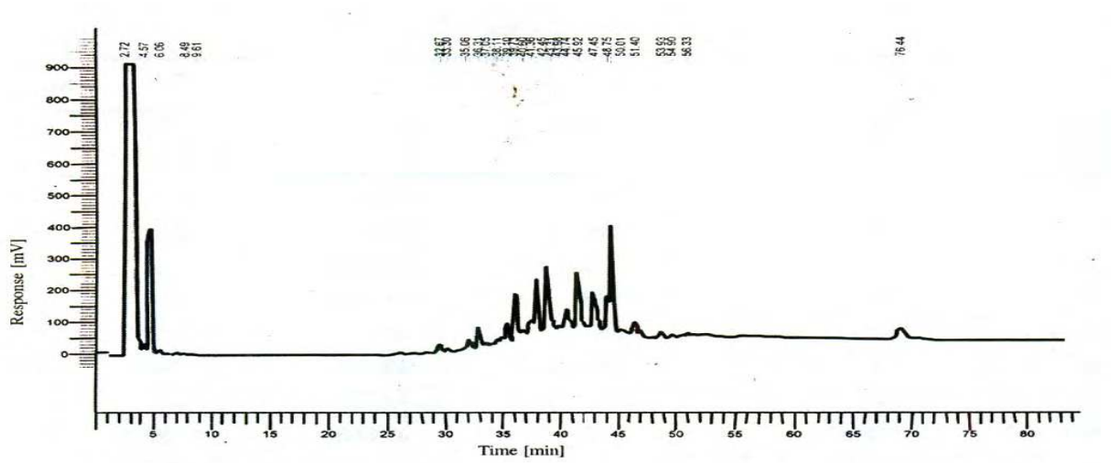

Plate 2. GC Chromatogram of Oil recovered after 2 months of placement of the sample 3B5.

Sample	Oil added	Amount of degraded oil (g) after				
Number	g	1 month	2 month	3 month	4 month	5 month
1B5	60	21.126	30.355	47.322	53.832	54.753
3B5	60	25.751	32.790	48.048	54.132	55.052

Table 17. Amount of Kerosene Oil Degraded against time in Months.

Gas Chromatographic Analysis of the extracted oil after 3 months of mixing in the Diesel oil polluted samples of 1C3, 2C3(Plate-3) and 3C3 gave 50, 42 and 27 peaks respectively (Sarma ,2010). It has been seen that the peaks at 1C3 starts at retention time 29.592 minute onwards, which for the 2C3 and 3C3 are 31.873 and 40.645 minute respectively.

Sl.	Sl No	1B5		3B5		Minute
No.	As in 1B5 Chromatogram	Retention Time (Minute)	Area %	Retention Time (Minute)	Area %	Delay in Ret. time
01	01	04.387	01.45	04.567	0.27	0.180
02	02	04.838	19.73	05.028	7.09	0.190
03	03	32.566	01.23	32.669	0.34	0.103
04	04	35.389	02.07	35.490	0.55	0.101
05	05	36.207	05.41	36.309	1.28	0.102
06	06	38.435	00.43	38.541	0.18	0.106
07	07	38.993	03.59	39.096	0.89	0.103
08	08	39.623	10.59	39.726	2.51	0.103
09	09	41.251	00.30	41.360	1.09	0.109
10	10	41.760	09.89	41.867	3.94	0.107
11	11	42.813	11.15	42.923	4.41	0.110
12	12	44.627	01.28	44.743	0.93	0.116
13	13	45.813	07.65	45.921	3.03	0.108
14	14	47.341	04.21	47.446	3.05	0.105
15	15	48.645	04.00	48.748	2.07	0.103
16	16	48.962	15.74	49.073	6.84	0.111
17	17	51.309	01.27	51.404	0.75	0.095

Table 18. List of Similar Peaks in GC Analysis Report of 1B5 and 3B5.

Sl. No.	Sl. No. of peak	Retention Time in minute	Area %	Sl. No.	Sl. No. of peak	Retention Time in minute	Area %
1	1	2.716	57.97	10	20	42.454	0.10
2	4	6.061	0.10	11	22	43.307	0.50
3	5	8.486	0.00	12	23	43.976	0.02
4	6	9.611	0.00	13	29	50.011	0.13
5	8	33.300	0.18	14	31	51.815	0.44
6	9	35.062	0.05	15	32	53.927	0.28
7	12	37.053	0.49	16	33	54.903	0.01
8	13	38.110	0.16	17	34	56.332	0.01
9	17	40.604	0.22	18	35	76.439	0.12

Table 19. List of peaks found in the sample 3B5 but not in 1B5.

Against 50 compounds present in 1C3, there are 42 and 27 compounds present in the samples 2C3 and 3C3, where nutrients and Hydrogen Peroxide were applied respectively. It implies that nutrients and Hydrogen Peroxide have a positive role in degradation of higher hydrocarbons into lower ones and subsequent removal of them from soil. A list of similar peaks in GC analysis report of 1C3, 2C3 and 3C3 are given in table (Table-20). It has been seen that the peaks due to same compounds appear in the chromatogram of 2C3 and 3C3 after an average delay in retention time of 0.078 and 0.123 minutes compared to the peaks of 1C3 in a range of 0.018 to 0.194 and 0.087 to 0.232 minutes respectively. This minute difference in retention time is probably due to minor change in experimental conditions during transit. There two peaks in the chromatogram of 2C3 and 3C3 at 54.466 and 54.552 minute with area % 0.53 and 0.22 respectively are probably due to same compound, which is a degradation product. The other two such compounds appear at retention time 61.654 and 71.422 minute in the 2C3 and 3C3 reports are also due to degradation products.

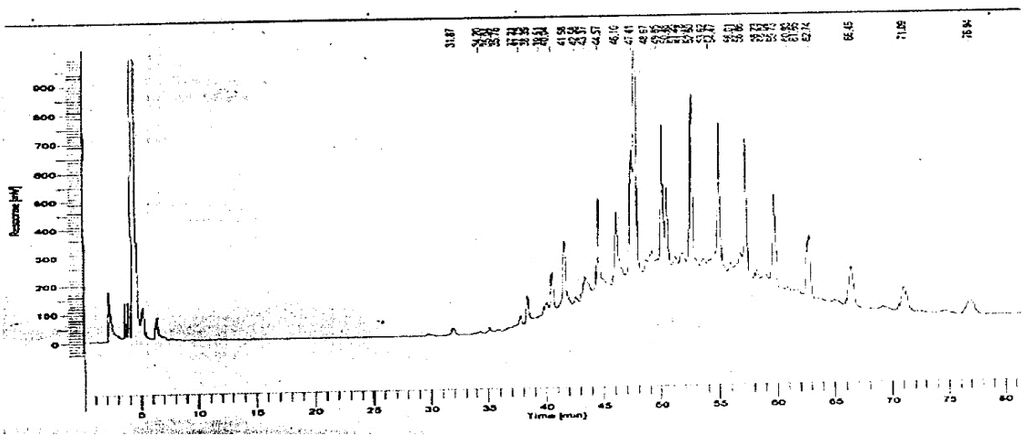

Plate 3. GC Chromatogram of Oil recovered after 3 months of placement of the sample 2C3.

In the GC report of the extracted oil samples from 1C5, 2C5 and 3C5 after three months of placement, number of peaks seen are 21, 34 and 40 respectively. From gravimetric analysis it has been found that extent of degradation in corresponding C5 samples are higher than C3 samples (Table-21). Thus it seems that number of components increases against increase of degradation at a certain stage and then the components gradually disappear. A list of peaks due to same compounds is given in table (Table-22). The peaks at 2C5 appear with a negligible average delay of 0.012 minute in retention time in the range of -0.036 to 0.055. The same for 3C5 peaks is 0.007 minute in the range of -0.070 to 0.034 minute. The existence of these compounds in all the samples indicates that these

compounds could resist degradation during the initial period of three months. The other compounds present in 2C5 and 3C5 are some of the degradation products. In the same way similar peaks have been found in 1C3 and 1C5, 2C3 and 2C5, and between 3C3 and 3C5.

The chromatograms obtained from the extracts of Lubricating oil polluted samples 1D1, 1D2 and 1D3 (Plate-4) are represented in plates. Number of components which persists degradation and number of components produced as a result of microbial degradation taken together has been found to be 64, 53 and 67 respectively, including solvent peak after lapse of 3 months (Sarma & Bhattacharyya ,2010). From the gravimetric analysis, it appears

-1- SlNo. (as per GC 1C3)	-2- 1C3 Retention Time (RT)	-3- 1C3 Area % of the peak	-4- 2C3 RT	-5- 2C3 Area % of the peak	-6- 3C3 RT	-7- 3C3 Area % of the peak	-8- Minute Delay in RT of 2C3 compared to 1C3	-9- Minute Delay in R T of 3C3 compared to 1C3
1	29.952	0.03	-	-	-	-	-	-
2	31.792	0.44	31.873	0.33	-	-	0.081	-
3	34.112	0.11	34.195	0.06	-	-	0.083	-
4	34.996	0.03	35.042	0.21	-	-	0.046	-
5	35.693	0.27	35.776	0.01	-	-	0.083	-
6	36.533	0.24	-	-	-	-	-	-
7	37.100	0.08	37.214	0.02	-	-	0.114	-
8	37.662	0.96	37.753	0.41	-	-	0.091	-
9	38.287	1.60	38.392	1.32	-	-	0.105	-
10	38.806	0.15	-	-	-	-	-	-
11	39.415	0.39	39.508	0.01	-	-	0.093	-
12	39.849	2.57	40.043	0.93	-	-	0.194	-
13	40.449	3.17	40.514	2.21	40.645	0.53	0.065	0.131
14	41.504	2.19	41.574	4.41	41.722	0.90	0.070	0.148
15	42.504	1.35	42.576	0.32	-	-	0.072	-
16	43.276	2.95	43.368	0.75	43.495	0.05	0.092	0.127
17	43.651	1.73	-	-	-	-	-	-
18	44.190	1.35	-	-	-	-	-	-
19	44.513	3.53	44.574	4.03	44.690	2.37	0.061	0.116
20	44.974	0.83	-	-	-	-	-	-
21	45.364	0.03	-	-	-	-	-	-
22	46.046	5.28	46.101	5.23	46.195	2.19	0.055	0.094
23	47.365	3.74	47.414	5.96	47.509	5.33	0.049	0.095
24	47.556	22.91	47.634	15.65	47.810	19.82	0.078	0.176
25	48.608	0.51	48.674	0.06	48.786	0.10	0.066	0.112
26	49.142	1.12	49.160	0.81	49.265	0.57	0.018	0.105
27	49.583	0.02	49.649	0.20	-	-	0.066	-
28	50.016	3.99	50.073	6.62	50.163	9.04	0.057	0.090
29	50.425	6.06	50.476	3.81	50.563	4.54	0.051	0.087
30	51.141	0.76	51.200	0.44	51.317	0.07	0.059	0.117
31	51.755	0.10	51.779	0.09	51.980	0.23	0.024	0.201
32	52.532	6.49	52.597	8.59	52.687	12.80	0.065	0.090
33	53.474	0.58	53.623	0.54	53.747	0.37	0.149	0.124
34	54.007	0.03	54.136	0.32	54.237	0.04	0.129	0.101
35	54.938	5.55	54.993	7.34	55.087	10.74	0.055	0.094
36	55.954	0.88	56.006	0.68	56.110	0.08	0.052	0.104
37	56.442	0.43	56.476	0.43	56.578	0.03	0.034	0.102
38	56.834	1.97	56.859	1.32	56.972	0.86	0.025	0.113
39	57.213	4.15	57.273	6.59	57.370	9.76	0.060	0.097
40	58.169	0.86	58.229	0.61	58.341	0.06	0.060	0.112
41	58.738	0.10	-	-	-	-	-	-
42	58.927	0.41	59.042	0.37	-	-	0.115	-
43	59.664	3.21	59.735	5.39	59.869	7.94	0.071	0.134
44	60.389	0.04	-	-	-	-	-	-
45	60.865	0.23	60.930	0.02	-	-	0.065	-
46	62.645	2.45	62.735	4.64	62.911	6.41	0.090	0.176
47	66.342	2.18	66.447	3.90	66.679	4.70	0.105	0.232
48	70.973	1.54	71.094	2.92	-	-	0.121	-

49	72.360	0.33	-	-	-	-	-	-
50	76.800	0.08	76.942	1.88	-	-	0.142	-

Table 20. List of Similar Peaks in GC Analysis Report of 1C3, 2C3 and 3C3.

Sample	Oil Added	Percent of the applied oil Degraded after				
No.	g	1 month	2 months	3 months	4 months	5 months
1C3	30	27.84	43.18	45.20	65.53	67.66
1C5	60	24.25	46.03	56.04	72.43	75.50
2C3	30	33.19	49.88	51.89	69.97	70.98
2C5	60	38.33	53.13	59.03	73.78	77.19
3C3	30	30.91	45.38	47.93	69.09	69.99
3C5	60	36.30	49.52	57.45	73.33	76.45

Table 21. Percent of Applied Diesel Oil Degraded against time in Months.

-1- Sl No. (as per GC report of 1C5)	-2- 1C5 Retention Time (RT)	-3- 1C5 Area % of the peak	-4- 2C5 RT	-5- 2C5 Area % of the peak	-6- 3C5 RT	-7- 3C5 Area % of the peak	-8- Minute Delay in RT of 2C5peaks compared to peaks of 1C5	-9- Minute Delay in R T of 3C5peaks compared to peaks of 1C5
1	0.986	0.00	-	-	-	-	-	-
2	35.087	0.77	35.051	0.81	35.017	0.65	-0.036	-0.070
3	37.761	0.83	37.759	0.71	37.753	0.63	-0.002	-0.008
4	38.426	3.84	38.411	3.22	38.395	2.60	-0.015	-0.031
5	40.075	0.36	40.059	1.48	40.034	1.33	-0.016	-0.041
6	40.525	2.94	40.530	2.76	40.526	2.51	0.005	0.001
7	41.595	7.77	41.595	6.44	41.589	5.82	0.000	-0.006
8	43.364	0.72	43.379	1.03	43.381	0.97	0.015	0.017
9	44.581	6.46	44.589	5.64	44.586	5.05	0.008	0.005
10	46.083	2.79	46.106	2.33	46.106	2.30	0.023	0.023
11	47.399	6.71	47.411	6.56	47.415	6.16	0.012	0.016
12	47.699	19.61	47.724	16.63	47.729	16.09	0.025	0.030
13	50.055	7.50	50.073	6.96	50.075	6.44	0.018	0.020
14	50.448	3.35	50.478	2.91	50.479	2.84	0.030	0.031
15	52.578	8.30	52.597	8.43	52.598	8.28	0.019	0.020
16	54.982	6.70	54.995	6.87	54.999	6.67	0.013	0.017
17	57.263	6.27	57.275	6.37	57.280	6.32	0.012	0.017
18	59.730	5.87	59.751	5.53	59.748	5.65	0.021	0.018
19	62.729	5.07	62.755	4.83	62.752	5.13	0.026	0.023
20	66.451	3.95	66.477	4.06	66.479	4.44	0.026	0.028
21	71.096	0.21	71.151	3.02	71.130	3.44	0.055	0.034

Table 22. List of Similar peaks in GC Analysis Report of 1C5, 2C5and 3C5.

that the extent of degradation is highest in the samples with nutrients. The extent of degradation in presence of hydrogen peroxide is moderate. It seems that hydrogen peroxide increases number of components and later these degradation products disappear from soil. There are some peaks which seem to be due to same compounds (Table-23).

-1- Sl No. (as per GC report of 1D1)	-2- 1D1 Retention Time (RT)	-3- 1D1 Area % of the peak	-4- 1D2 RT	-5- 1D2 Area % of the peak	-6- 1D3 RT	-7- 1D3 Area % of the peak	-8- Minute Delay in RT of 1D2 peaks compared to 1D1	-9- Minute Adv in R T of 1D3 peaks compared to peaks of 1D1
1	8.569	0.06	-	-	8.340	0.03	-	0.229
2	9.381	2.03	9.706	1.07	9.107	4.32	0.325	0.274
3	11.562	21.09	11.822	18.96	11.316	10.34	0.260	0.246
4	12.595	0.47	-	-	12.363	0.43	-	0.232
5	13.203	0.09	-	-	12.912	0.55	-	0.291
6	14.122	0.77	14.367	0.37	13.865	0.76	0.245	0.257
7	14.792	0.51	15.026	0.29	14.578	0.47	0.234	0.214
8	15.823	0.23	16.056	0.14	15.535	0.10	0.233	0.288
9	16.167	0.42	16.397	0.27	15.896	0.18	0.230	0.271
10	17.268	0.16	17.541	0.07	16.934	0.67	0.273	0.334
11	18.339	0.18	18.584	0.14	18.052	0.16	0.245	0.287
12	18.714	2.08	18.933	1.54	18.405	3.96	0.219	0.309
13	19.484	7.78	19.689	6.89	19.080	8.69	0.205	0.404
14	19.989	11.70	20.220	11.26	19.667	10.63	0.231	0.322
15	20.761	0.25	-	-	-	-	-	-
16	21.742	0.65	21.973	0.42	21.440	1.29	0.231	0.302
17	22.356	10.06	22.557	8.57	21.920	12.71	0.201	0.436
18	24.520	0.18	-	-	24.165	0.45	-	0.355
19	24.787	0.30	24.985	0.28	24.494	0.66	0.198	0.293
20	27.586	0.28	27.782	0.33	27.260	1.03	0.196	0.326
21	28.222	11.95	28.402	12.15	27.867	8.89	0.180	0.355
22	28.979	0.75	29.143	0.84	28.683	0.75	0.164	0.296
23	29.300	0.68	29.472	0.81	28.996	0.78	0.172	0.304
24	30.794	1.50	30.980	1.14	30.466	3.20	0.186	0.328
25	31.355	1.61	31.609	1.14	30.890	3.71	0.254	0.465
26	34.324	0.29	34.492	0.16	33.982	0.33	0.168	0.342
27	36.155	0.18	36.310	0.12	35.804	0.33	0.155	0.351
28	37.154	0.02	-	-	36.700	0.14	-	0.454
29	38.152	1.27	38.311	0.89	37.798	2.15	0.159	0.354
30	39.031	0.06	-	-	38.677	0.04	-	0.354
31	40.092	0.11	40.246	0.04	39.770	0.06	0.154	0.322
32	40.681	0.32	40.828	0.13	40.416	0.01	0.147	0.265
33	42.504	0.54	42.645	0.10	42.133	0.41	0.141	0.371
34	42.818	0.35	42.963	0.06	42.799	0.07	0.145	0.019
35	43.996	2.55	44.169	1.07	43.681	1.98	0.173	0.315
36	44.518	1.35	44.668	0.62	44.154	2.03	0.150	0.364

37	45.121	0.35	-	-	44.736	0.24	-	0.385
38	45.709	0.13	45.904	0.03	-	-	0.195	-
39	46.872	1.25	47.021	0.49	46.464	0.11	0.149	0.408
40	48.391	0.48	48.549	0.12	-	-	0.158	-
41	49.701	1.68	49.842	0.72	-	-	0.141	-
42	50.014	1.40	50.160	0.52	-	-	0.146	-
43	51.005	0.12	51.126	0.02	-	-	0.121	-
44	51.342	0.33	51.505	0.32	-	-	0.163	-
45	52.366	1.60	52.523	0.60	-	-	0.157	-
46	52.771	0.82	52.920	0.39	-	-	0.149	52.771
47	53.531	0.29	53.715	0.09	-	-	0.184	53.531
48	54.260	0.44	54.469	0.12	-	-	0.209	54.26
49	54.891	1.82	55.049	0.55	54.482	0.03	0.158	0.409
50	55.294	0.19	-	-	55.108	0.06	-	0.186
51	55.933	0.24	56.121	0.08	55.492	0.33	0.188	0.441
52	56.423	0.03	-	-	-	-	-	-
53	56.735	0.02	-	-	-	-	-	-
54	57.294	2.09	57.477	0.90	57.003	0.52	0.183	0.291
55	58.369	0.03	58.555	0.01	-	-	0.186	-
56	58.872	0.02	-	-	-	-	-	-
57	59.253	0.02	-	-	-	-	-	-
58	59.864	1.07	60.083	0.35	-	-	0.219	-
59	61.138	0.02	61.187	0.31	-	-	0.049	-
60	62.107	0.03	-	-	-	-	-	-
61	62.978	1.12	63.260	0.25	-	-	0.282	-
62	66.811	0.70	67.215	0.02	-	-	0.404	-
63	71.637	0.87	-	-	-	-	-	-
64	77.684	0.01	-	-	-	-	-	-

Table 23. List of Similar Peaks in GC Analysis Report of 1D1, 1D2 and 1D3.

It means that these common compounds are the compounds which are resistant to degradation. The number of such compounds in between 1D1 and 1D2 is 48, between 1D1

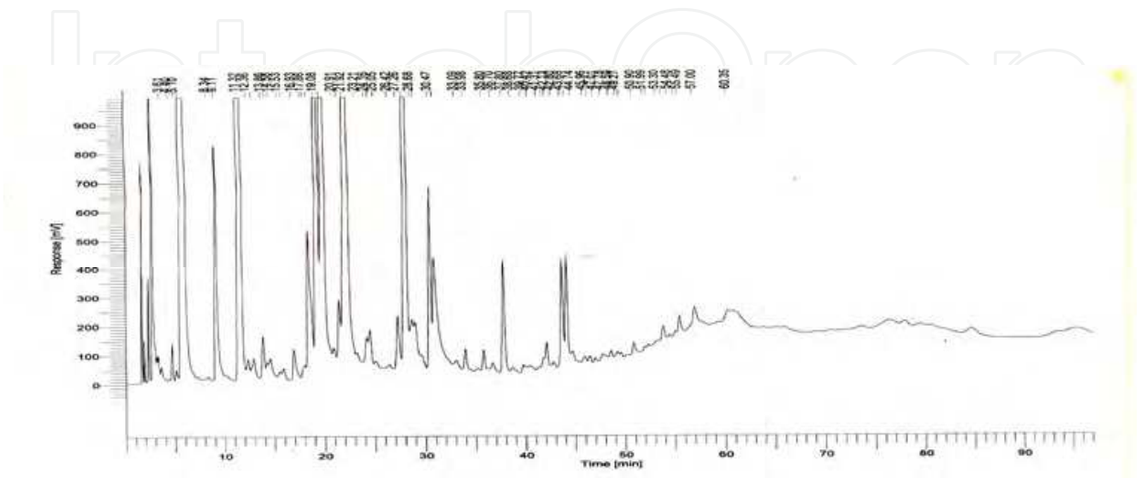

Plate 4. GC Chromatogram of oil recovered after 3 months of placement of sample 1D3.

and 1D3 is 41and among the three is 33 .The common compounds appear with a uniform difference in their retention time. 1D2 peaks appear with an average delay of 0.194 minute in the range of 0.049 to 0.404 minute and 1D3 peaks appear with an average advance retention time of 0.318 in the range of 0.019 to 0.441 minute. The other peaks of 1D2 and 1D3 are probably due to some degradation products. Number of such compounds in 1D2 is 5 and in 1D3 are 26. It indicates that Hydrogen Peroxide decomposes the higher hydrocarbons first and then the degraded components gradually disappear.

G.C. analysis of the recovered oil from residual oil polluted sample 1E5 after 1,2 (Plate-5) and 3 months gave 37, 65 and 52 peaks due to the undegraded components, derivatives and degradation products respectively. Out of the 37 components obtained after 1 month; 33 and 29 could resist degradation in the second and third months respectively, as revealed from their peaks appeared at a uniformly different retention time(Sarma et al,2004a) (Table-24). The stated 33 peaks appeared at a uniform advance retention time in the range of 0.914 to 1.247 minute with an average of 1.046. The stated 29 peaks appeared at a uniform advance retention time in the range of 0.914 to 1.247 minute with an average of 1.233. The remaining 4 and 4 components appeared in chromatograms obtained after 1 and 2 months respectively seems to be lost from the soil due to microbial activities. The number of peaks appeared in the chromatogram taken after 2 months but not in the previous chromatogram are 32. These peaks, and the corresponding peaks present in the last chromatogram are given in table (Table -25). Here only 17 peaks could resist complete degradation. These 17 peaks appeared at a uniform advance retention time in the range of 0.038 to 0.403 minutes with an average advance retention time of 0.196 minutes. The other 15 components seem to be lost from the soil due to microbial activities during the third month. Out of total 52 peaks appeared in the last chromatogram, only 6 peaks are due to new compounds (Table-26). These are the degradation products of the oil in the third month. Thus it can be concluded that degradation in the initial stage is more vigorous. The process slows down gradually. During degradation the higher compounds produce some fragments or some derivatives as a result of which number of components increases. Later on these compounds gradually disappear.

Table-27 shows number of components detected by GC analysis of a few samples of some of the mineral oils viz. Crude oil, Kerosene oil and Diesel oil–without agents, with nutrients, and with hydrogen peroxide. It appears that Hydrogen Peroxide increases the number of components in the sample and thus degraded the higher compounds. Application of NPK also increases the same, but lesser than Hydrogen Peroxide. For example, in the A5 samples, number of components after 3 months becomes 22 in 1A5 (without agent), 37 in 2A5 (with nutrients) and 39 in 3A5 (with hydrogen peroxide).The following table (Table 28) shows number of components detected by GC analysis of a few samples under identical conditions after one, two and three months. It appears that number of components increases initially and then decreases. It means that higher compounds give some smaller compounds and then escape from soil.

A good number of common components can be identified in the GC chromatogram of different samples (Table-29). These common components appear in the chromatogram after maintaining a uniform difference in their retention time. This difference is probably due to minor difference in the experimental conditions. In between the peaks at serial number 1and 2, the average difference in the retention time is 1.311 minute in the range of 1.304 to 1.316minute. Similarly the average difference in retention time between peaks at serial

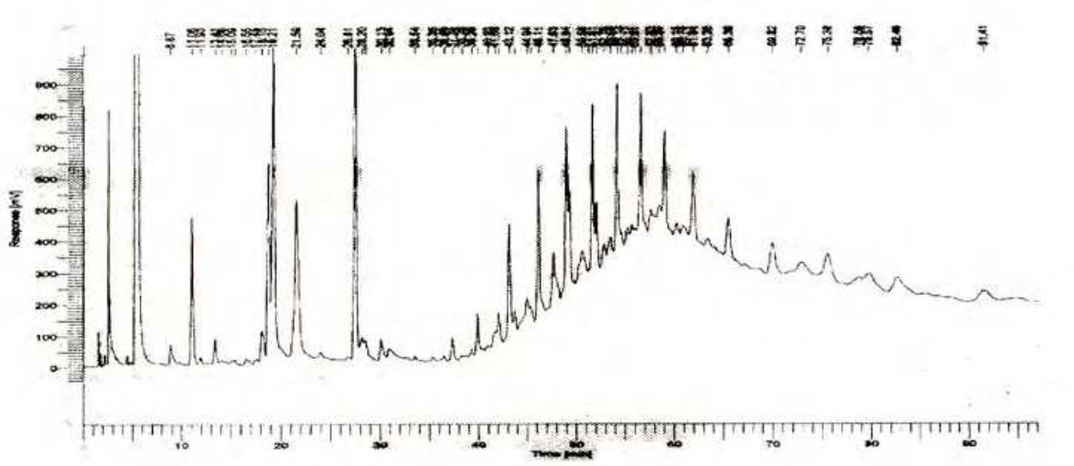

Plate 5. GC Chromatogram of Oil recovered after 2 months of placement of the sample 1E5.

-1- Sl No.	-2- After 1 month	-3- After1 month	-4- After 2 months	-5- After 2 months	-6- After3 month	-7- After 3 months	-8- Minute	-9- Minute
(as per GC report of analysis after 1 month)	Retention Time (RT)	Area % of the peak	RT	Area % of the peak	RT	Area % of the peak	Advance in RT of peaks after 2 months compared to peaks after 1 month.	Advance in Retention Time of peaks after 3 months compared to peaks after 1 months.
1	09.789	0.70	08.875	0.66	08.619	1.95	0.914	1.170
2	11.995	1.40	11.058	4.21	10.870	2.17	0.937	1.125
3	14.371	0.55	13.409	0.72	13.220	0.55	0.962	1.151
4	19.036	1.45	18.154	1.13	17.819	2.67	0.969	1.304
5	19.728	8.97	18.759	5.48	18.565	11.79	0.969	1.163
6	20.263	19.26	19.212	10.73	18.998	17.44	1.051	1.265
7	22.609	10.82	21.591	7.81	21.390	15.79	1.018	1.219
8	28.498	5.98	27.418	11.07	27.223	17.21	1.080	1.275
9	31.191	1.02	30.128	0.77	29.860	2.48	1.063	1.331
10	32.031	0.12	30.941	0.46	30.548	2.95	1.090	1.483
11	38.424	0.84	37.337	0.65	37.159	1.89	1.087	1.265
12	40.941	0.60	39.910	1.02	39.739	0.10	1.031	1.202
13	43.073	0.32	42.042	1.02	-	-	1.031	-
14	44.147	2.36	43.117	3.73	43.058	1.56	1.030	1.089
15	44.768	0.55	43.702	0.78	43.562	1.03	1.066	1.206
16	46.013	0.17	44.935	1.80	44.782	0.08	1.078	1.231
17	47.132	2.25	46.111	4.47	45.936	0.96	1.021	1.196
18	48.652	0.54	47.627	0.94	47.471	0.20	1.025	1.181
19	49.956	2.89	48.942	4.54	48.767	1.49	1.014	1.189
20	50.267	2.10	49.252	2.81	49.078	0.91	1.015	1.189

21	51.235	0.13	-	-	-	-		
22	51.630	0.47	50.560	0.95	50.339	0.40	1.070	1.291
23	52.626	2.78	51.610	4.47	51.439	1.42	1.016	1.187
24	53.023	1.26	52.005	1.81	51.829	0.62	1.018	1.194
25	53.820	0.19	52.760	0.75	52.632	0.33	1.060	1.188
26	54.538	0.34	53.444	0.99	53.248	0.53	1.094	1.290
27	55.148	2.61	54.134	4.84	53.964	1.42	1.014	1.184
28	56.205	0.41	55.165	0.42	54.955	0.82	1.040	1.250
29	56.695	0.02	55.642	0.32	-	-	1.053	-
30	57.554	2.85	56.534	3.53	56.375	1.46	1.020	1.179
31	58.662	0.04	57.531	0.36	57.353	0.26	1.131	1.309
32	60.198	1.80	58.951	2.97	58.747	1.18	1.247	1.451
33	61.305	0.58	60.145	0.42	-	-	1.160	-
34	63.399	1.66	-	-	-	-	-	-
35	67.374	1.17	-	-	-	-	-	-
36	72.364	0.10	-	-	-	-	-	-
37	80.707	20.73	79.568	0.03	-	-	1.139	-

Table 24. List of Similar Peaks in GC analysis report of 1E5 after 1,2 and 3 months.

number 2 and 3, 3 and 4, 4 and 5, and between 5 and 6 are 0.319 (0.300 to 0.351), 2.355 (2.347 to 2.369) , 0.397 (0.386 to 0.416), and 2.113 minute (in the range of 2.084 to 2.130 min) respectively. It clearly indicates that these are the compounds which are resistant to egradatdion till the period of their extraction from soil.

-1-	-2-	-3-	-4-	-5-	-6-	-7-
	Sl No.	2 months	2 months	3 months	3 months	Minute
Sl No.	(as per GC report of analysis after 2 months)	Retention Time (RT)	Area % of the peak	Retention Time	Area % of the peak	Advance in RT of peaks after 3 month compared to peaks after 2 months
1	3	11.929	0.17	11.737	0.15	0.192
2	5	14.064	0.05	13.880	0.01	0.184
3	6	15.094	0.07	-	-	-
4	7	15.428	0.12	-	-	-
5	8	16.554	0.08	16.332	0.25	0.222
6	9	17.450	0.02	17.240	0.03	0.210
7	14	24.038	0.17	23.874	0.45	0.164
8	15	26.806	0.05	26.636	0.52	0.170
9	17	28.199	0.70	28.021	1.42	0.178
10	18	28.520	0.62	28.353	1.42	0.167
11	21	33.537	0.11	33.350	0.23	0.187
12	22	35.357	0.09	35.177	0.29	0.180
13	23	36.488	0.06	-	-	-

14	25	38.282	0.06	-	-	-
15	26	39.289	0.15	39.171	0.05	0.118
16	28	40.934	0.08	-	-	-
17	29	41.694	0.51	41.556	0.31	0.138
18	34	45.297	0.60	-	-	-
19	40	51.187	0.02	-	-	-
20	46	54.510	0.28	54.472	0.02	0.038
21	49	55.912	0.08	-	-	-
22	52	58.387	0.61	58.164	0.14	0.223
23	55	60.775	0.10	-	-	-
24	56	61.840	2.49	61.585	0.95	0.255
25	57	63.256	0.33	-	-	-
26	58	65.392	1.83	65.082	0.82	0.310
27	59	69.823	1.82	69.420	0.82	0.403
28	60	72.697	0.06	-	-	-
29	61	75.388	1.82	-	-	-
30	62	78.565	0.03	-	-	-
31	64	82.460	0.09	-	-	-
32	65	91.411	0.07	-	-	-

Table 25. List of Similar Peaks due to Degradation Products in GC Analysis Report of 1E5 after two and three months.

In order to make an attempt to identify different components those are persistent for a stipulated time in the soil samples, an experiment with some standard sample solutions were done (Sarma & Devi, 2009). Soil samples having no background of oil pollution were taken as per the following table (Table-30).

Sl No.	Sl No. (as per GC report)	After 3 months Retention Time (minute)	After 3 months Area % of the peak
1	8	17.471	0.03
2	22	36.086	0.07
3	43	56.823	0.07
4	47	59.522	0.16
5	51	74.861	0.04
6	52	76.556	0.08

Table 26. Peaks of compounds found after three months in 1E5 but not earlier.

Sl. No	Sample	Number of Components detected		
		Without Agent (first set)	With Nutrient (second set)	With H_2O_2 (third set)
1	A5(Crude Oil)	22	37	39 (plate-1)
2	B5(K Oil)	17	30	35(Plate-2)
3	C5(Diesel Oil)	21	34	40

Table 27. Number of Components detected after same time interval in Soil.

Extract of different samples by GC Analysis.

Sl. No.	Sample	Number of Components detected		
		After one month	After two months	After three months
1	2A3	17	24	23
2	3A3	11	33	32
3	1E5	37	65 (Plate-5)	52

Table 28. Number of Components detected after uniform time gap in Soil Extract of different samples by GC Analysis.

Laboratory temperatures during the experiment were in the range of 12.8 to 36.4^{0}C. The Physicochemical parameters of this soil sample are as given below (Table-31). These were suitable for the process of bioremediation to occur. The amount of recovered oil is 18046, 33638 and 51250 ppm in the samples S1, S2 and S4 respectively. It shows that the nutrients and Hydrogen Peroxide have expedited the process of oil degradation. The recovered oil from S2, S4 and some common known aromatic compounds in n-hexane were GLC analysed. The n-hexane soluble parts of the recovered oil exhibited peaks in the retention time of 39.006, 39.971, 40.118, 41.615, 41.656, 44.070, 44.151 and 62.596 minutes in the GC chromatograms. The GC peaks of the known compounds are as in Table 32A and B.

1-Naphthol and 2-Naphthol exhibited their peaks at retention time range of 37.095 to 37.238 minutes and 36.894 to 37.018 minutes averaging 37.184 ± 0.089 minutes and 36.969 ± 0.075 minutes respectively in different chromatograms. The lack of peaks before retention time of 39.066 minutes in the oil sample indicates that 2-Naphthol and 1-Naphthol are not present

Serial Number		Selection of common components from GC Chromatogram of Samples				
		1A5 -2 m	1B5- 2 m	1C5-3m	1D3-3m	1E5-2m
1	X	48.772	47.341	46.083	-	47.627
	Y	1.85	4.21	2.79	-	0.94
	Z	-	-	-	-	-
2	X	-	48.645	47.399	49.268	48.942
	Y	-	4.00	6.71	0.11	4.54
	Z(1.311)	-	1.304	1.316	-	1.315
3	X	50.389	48.962	47.699	49.619	49.252
	Y	23.34	15.74	19.61	0.07	2.81
	Z(0.319)	1.617*	0.317	0.300	0.351	0.310
4	X	52.736	51.309	50.055	51.988	51.610
	Y	0.27	1.27	7.50	0.04	4.47
	Z(2.355)	2.347	2.347	2.356	2.369	2.358
5	X	53.152	-	50.448	52.374	52.005
	Y	4.70	-	3.35	0.06	1.81
	Z(0.397)	0.416	-	0.393	0.386	0.395
6	X	55.236	-	52.578	54.482	54.134
	Y	2.48	-	8.30	0.03	4.84
	Z(2.113)	2.084	-	2.13	2.108	2.129

* = 1.307 +0.310 = 1.617

Table 29. List of Peaks due to Common Components in all the types of oil pollutants.

in here, X = Retention Time in minute in the chromatograms, Y = Area Percent in the Chromatograms, Z = Difference in Retention Time with the previous peak of the same sample in the chromatogram reported in the table. The figure within parenthesis ( ) indicates average difference in Retention Time of all the types of oil.

Sample No	Mass of Soil Taken(g)	Crude Oil Concentration (ppm)	Emulsifier added (mL)	NPK added (g)	H_2O_2 added (mL)	Water added (mL)
S 1	2910	20,000	10	30	-	100
S 2	2940	20,000	10	-	30	100
S 3	3000	0	10	-	-	100
S 4	2940	20,000	10	-	-	100

Table 30. Composition of Experimental Samples.

Physical Parameters			Chemical Parameters		
Name	Result	Unit	Name	Result	Unit
Texture	Sandy Loam	-	pH	6.87	-
Electrical Conductivity	0.0137	$mScm^{-1}$	Soil Org Carbon	1.41	%
Hydraulic Conductivity	$1.73X10^{-2}$	$cm\ s^{-1}$	Nitrogen	0.11	ppm
Water Holding Capacity	25.56	%	Phosphorous	0.09	ppm
Porosity	48.8	%	Potassium	0.92	ppm

Table 31. Physicochemical parameters of the soil sample.

Sl No	Compound	Sl No	Compound	Sl No	Compound
1	2-Naphthol	5	Naphthalene	9	Benzil
2	1- Naphthol	6	Anthracene	10	Phthalic Acid
3	Benzophenone	7	Benzoic Acid		
	Cinnamic Acid	8	Benzoin		

Table 32A. Compound Serial Numbers Used in the Table 32B.

Soln. No	Compounds Serial Number and their peaks with Retention Time ion Minutes									
	1	2	3	4	5	6	7	8	9	10
5				40.443						
6		37.173								51.856
7	37.018									
8							44.363			
9						40.786				
10	36.894	37.231			40.161		44.374			
11	36.996	37.095				41.243		44.910		
12			39.452				44.350		50.379	
13								44.998		
14			39.865					44.990		
15		37.238								

Table 32B. GC peaks of the known samples and their retention time.

On the other hand, lack of peaks around 44.9 minute, 50.3 minute and 51.8 minute in the oil sample chromatogram indicates that Benzoin, Benzil and Phthalic Acid are not present in the soil sample. The oil sample peaks at 39.971 minute, 40.118 minute, 41.615 minute, 41.656 minute, 44.070 minute and 44.157 minutes are very close to known sample peaks of Benzophenone, Cinnamic Acid, Anthracene and Benzoic Acid respectively. Out of these Anthracene and Naphthalene are tricyclic and bicyclic aromatic hydrocarbons respectively and others are oxygenated derivatives. The presence of these compounds in the polluted soil samples cannot be ruled out. That the polycyclic aromatic hydrocarbons, which are of great concern due to their toxicity and suspected carcinogenicity; are resistant to biodegradation was reported by many workers at different point of time.

The influence of applied phosphorous on bioremediation is positive. Since the phosphorous cycle is a sedimentary one, its fixation rate was studied. It has been found that a sample of sandy loam which possess 1.987µg g^{-1} available phosphorous fixes 99.85% phosphorous against addition of 50 g and 100 g of commercial single super phosphate fertiliser during progressive remediation from Lubricating oil pollution in a 120 days experiment(Sarma et al, 2008).

Bioremediation improves the soil physical conditions of petroleum polluted soil. During such remediation the amount of Soil Organic Carbon remarkably increases. A maximum of 111.51% and 65.20% increase of SOC was found in two samples of Sandy Clay and Sandy soil at an applied concentration of 20,000 ppm crude oil pollutant in an indoor experiment of 346 days. The increase of SOC in the samples where degradation was carried out in presence of added NPK was less and in those with added Hydrogen Peroxide was more than in the samples without NPK and Hydrogen Peroxide (Sarma & Sadhanidar , 2007).

It has been found that the pH and Electrical Conductivity of a remediating soil decreases. For example, in the first 6 months in the 1A5 sample pH decreases from 6.86 to 6.77; 6.73; 6.64; 6.59; 6.40 and 6.14 in regular monthly interval. Similarly, the EC decreases from 0.43 mS cm^{-1} to 0.41; 0.30; 0.27; 0.18; 0.14 and 0.11 mS cm^{-1} respectively in regular monthly intervals(Sarma et al, 2003a). Similar results were found when Lubricating oil is the pollutant (Sarma et al, 2003b, 2004b, 2005b). This is due to formation of some oxygenated derivatives from hydrocarbons, which are weakly acidic as the microbial degradation is a process of oxidation.

6. Conclusion

It has been found that under identical conditions a suitable soil sample degrades petroleum fractions to different extent. The extent of degradation of kerosene oil is highest, followed by crude oil, diesel oil, lubricating oil and residual oil. The disappearance of hydrocarbon is more in the initial stage and gradually it becomes a slow process. Complete recovery from hydrocarbon pollutants is not achieved during the experimental period of one year. The application of nutrients expedites the process of degradation. The action of hydrogen peroxide is moderate. The number of compounds in the recovered oil increases up to a period and then gradually decreases. In most cases the number of components generated is more in samples where hydrogen peroxide is applied. A good number of peaks formed probably due to same compounds can be pointed out and these appear in the chromatogram by maintaining an almost uniform difference in retention time. Parameters pH and Electrical

Conductivity show a decreasing trend on increase of degradation of the applied oil. Parameters Water Holding Capacity and Bulk Density become lower towards the side where presence of pollutants is higher. Parameters such as Hydraulic Conductivity and Organic Carbon become higher towards the side where presence of pollutants is higher. A good number of GC peaks in each of the samples are significant. The peaks so identified might be due to same components in the experimental oil. Since there is no probability of having such large number of common constituents in the oil samples; some of these peaks seem to be due to some degradation products.

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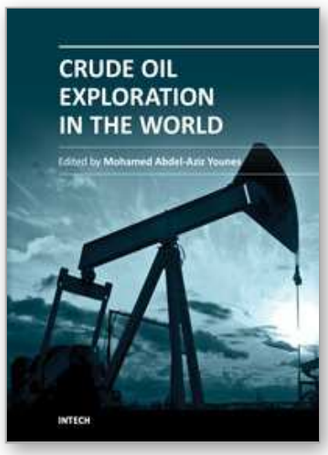

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