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Growth Responses of *Lens esculenta* Under Petroleum Contaminated Soil in Field and Pot Experiments

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Refinery effluent
Hydrocarbons
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Peak-height ratio method

ABSTRACT

The present investigation deals with the effect of different concentrations of refinery effluent on seed germination and seedling growth of *Lens esculenta*. The seeds were grown in pots and field and treated with various concentrations of refinery effluent. The plant showed positive effect on vegetative growth at lower concentration of the added refinery effluent, whereas higher concentration of refinery effluent showed gradual decrease in vegetative growth. The impact of petroleum refinery effluent irrigation on soil properties has been included in detail.

INTRODUCTION

The petroleum refineries directly discharge their effluents into the agro fields as land treatment method (Shailubhai 1986). Their obnoxious pollutants are known to be carcinogenic, mutagenic, xenobiotic and recalcitrant (Chet & Mitchell 1976, Atlas 1981). They are also known to affect the microbial systems and agricultural crops in terms of their germination ability, biochemical contents, disease resistance, deficiencies and yield like many others industrial effluents as studied by Chauhan & Kumar (1995), Baakah et al. (1997) and Dutta (1999). The deliberate discharge of industry effluents in agroecosystems deleteriously affects the quality and yield of leguminous crops (Leyval & Binet 1998).

The literature survey shows that microbiological degradation of petroeffluents is continuously worked upon by scientists but scanty work is done on the impact of oil pollution on crops having a symbiotic and asymbiotic microflora. A study on effect of various concentrations of effluents from two tanneries of Agra on total phenolics and isoperoxidases, isoenzymes and isoacidphosphatases in onion (*Allium cepa*) roots was undertaken by Chauhan & Kumar (1997). They reported that quality of total phenolics in the roots increased with the increase in the effluent concentration. The isozyme study made by starch gel electrophoresis exhibited marked reduction in the significant increase in the number of isoperoxidases indicating high pollution load in the tannery effluent.

Petroleum refinery generates a large volume of oil sludge, organics like n-alkanes-paraffins, olefins, aromatics, asphaltics, phenols and polynuclear aromatic hydrocarbons (Atlas 1981, Grob 1983), which have both lethal and sublethal effects on a wide variety of organisms (Chet & Mitchell 1973), reduced photosynthetic rate in algae (Parker & Menzel 1974), reduced resistance to environmental stress in crabs and accumulation of hydrocarbons in fatty tissues of fishes which get transferred to higher trophic levels including humans (Shailubhai 1986). Therefore, the concern over chronic hydrocarbon inputs in environment is ecologically mandatory and disposal of oil sludges and waste hydrocarbon is a major challenge prevailing in the petroleum industry.

Many prior attempts have been made to assess the characteristics of petroleum refinery wastes (Kale et al. 1985) and all reports suggest that the researches for regular assessment of this waste in order to obtain judicious data before they are used for irrigation, are much required (Shailubhai 1986, Bhadauria 1991).

MATERIALS AND METHODS

The experiment on *Lens esculenta* was conducted in the experimental field developed for this purpose, adjacent to the drain carrying treated effluent on Delhi-Agra Highway No. 2 in a split plot design where main plot treatments were irrigated with effluent discharged from the refinery. Other experiment on *Lens esculenta* was also conducted in pots in the Raja Balwant Singh College, Agra.

Analysis of effluent and soil samples: The refinery effluent samples were collected using bottle sampler from the middle part of the drain and transferred to clean 2.0 L airtight plastic containers, previously rinsed with drain water of the sampling point (APHA 1975).

The soil samples were collected by metallic soil borer. It was cleaned with absolute alcohol and plunged into the soil to a depth of 6 inches and slowly taken out. Soil collected was gently transferred into polythene bags which were immediately sealed over the flame to prevent the loss of moisture. From each locality 10 samples were collected and mixed to form a composite sample.

All the samples of soil and effluent were brought to the laboratory in ice-box and stored in a refrigerator at 4±1°C till the analysis was completed. Effluent samples were analysed for their physico-chemical and microbiological characteristics by standard methods (APHA 1975). The physical parameters included colour, odour, temperature, pH, conductivity and turbidity. Chemical parameters were dissolved oxygen, biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonical nitrogen, organic nitrogen, nitrate nitrogen, nitrite nitrogen, total grease and oil. The microbiological status of the samples was found by estimating total bacterial count and total fungal count using serial dilution method (Johnson 1957).

The soil samples were analysed for colour, temperature, pH, pore space distribution, bulk density, effective soil depth, odour, texture, water holding capacity, electrical conductivity, moisture content, nitrogen, humus, total organic carbon, total grease and oil, and total bacterial and fungal count using standard methods given by Pandeya et al. (1968). Hydrocarbons were determined by gas chromatography. The hydrocarbons of both soil and effluent were extracted in CCl_4. The extract was concentrated by evaporation at 50°C for 1 hour to 2.0 mL. 0.5 mL extract was injected in the gas chromatograph for the analysis using flame ionization detector (FID) and OV-1 stainless steel column.

The chromatograms were examined and the identification of different carbons was made by comparing with known hydrocarbon mixture separated under the identical operational conditions. The quantitative analysis was done by peak-height ratio method (Blumer et al. 1970) following the formula given below:

Peak Area = height of peak × width of half height of peak.

$$\text{Retention} = \frac{\text{Total distance travelled by solvent}}{\text{Chart speed}}$$

Impact of irrigation with petroleum refinery effluent on growth of test crops and soil properties in field and pot experiments: Seeds of test crop *Lens esculenta* were surface sterilized by immersing in acidified sodium hypochlorite solution for 20 minutes followed by thorough washing in distilled water. After sterilization of seeds, they were sown at the rate of 30 kg/ha. Irrigation by refinery effluents in agricultural soil was performed in experimental farm, Khandari campus, R.B.S. College, Agra. The effluents were brought in carbuoys and dilutions were prepared, viz., 1%, 0.5%, 0.1%, and 0.05% respectively and one control plot was irrigated by tube well water. The same dilutions of diesel (as it contains the whole spectrum of refinery waste) were used in pot experiment. The impact of hydrocarbons was studied on germination, plant root and shoot growth, and soil properties at different intervals.

RESULTS

Physico-chemical analysis of refinery effluent: The refinery wastes normally possessed light yellow colour but on dilution the effluent showed no visible colour (Table 1). Effluent gave oily odour but at 0.05% dilution, such odour was not observed. The temperature of the refinery effluent was 30.5°C but on dilution it was reduced to 26°C. The temperature varied according to the percentage of dilution. The pH of effluent was on alkaline side, i.e., 8.50. The pH target according to the percentage of water dilution was lowest being 7.5. The conductivity of effluent was 1.95 mmhos/cm, and at 1% dilution 1.24 mmhos/cm. At 0.5% it was 1.26 mmhos/cm, at 0.1% 1.12 mmhos/cm, and at 0.05% 1.04 mmhos/cm. The turbidity of the effluent was 72.0 CTU. The turbidity varied according to the percentage of water added to it. The dissolved oxygen of the refinery effluent was observed to be 4.90 mg/L. Total organic carbon of refinery effluent was 45.8 mg/L. As evidenced from the result BOD showed maximum value of 44.0 mg/L. The COD of the effluent showed the maximum value of 162.0 mg/L. Total nitrogen of the effluent was 1.66 mg/L but on dilution it was lowest at 1.19 mg/L. The gas chromatographic analysis revealed that the main pollutants in oil refinery effluent were saturated and unsaturated aliphatic hydrocarbons and a few unknown ones. In effluent, 15 hydrocarbons were recorded. The gas chromatogram and qualitative analysis of hydrocarbons are shown in Fig. 1 and Table 3. Only those gas chromatograms are shown and described which revealed the maximum information. Over all 12 chromatographic peaks corresponding to n-alkanes of carbon length C-12 to C-22 were encountered. Besides, alkenes C-13 to C-15 and few unknown hydrocarbons were also noticed.

Physico-chemical properties of soil of experimental site are given in Table 4. Pore space and bulk density are inversely related. The noncapillary porosity, in the plough zone is usually 10-30% (differ from crop to crop). In this field it was found to be 24%. The bulk density of highly productive and normal soil usually range from 1.1 to 1.5g/cm³ (medium to fine texture) and 1.2 to 1.65 g/cm³ (coarse texture). This soil possessed bulk density of 1.60g/cm³. Generally, excellent yields are produced on normal soil having an effective root zone depth from 45 to 90 cm. Effective soil depth for this crop was found to be 53-59 cm. Since long farmers have been using the colour as an index of soil fertility. The colour of the soil was brown with blackish humus. Effluent gave off oily and phenolic odour. Initially the soil had natural muddy odour but after addition of irrigant this odour changed to oily and phenolic sting. Oily odour was observed in the plots with 1% and 0.5%, and mild oily odour was observed in 0.05% and 0.1%. Soil texture consisted of sand 58.34%; slit 17.55% and clay 30.15% at the study site. Water holding capacity represents the maximum limit of moisture, which a soil can hold when fully saturated with water. It was averagely 36.64. The soil temperature at experimental

Table 1: Physio-chemical characteristics of petroleum refinery effluent before and after dilution. (LY = Light Yellow, OF = Oily Ferrous).

S.No.	Parameters	0.05%	0.10%	0.50%	1%	Initial
1	Colour	Nil	Nil	Nil	LY	LY
2	Odour	NO	NO	NO	OF	OF
3	Temperature (°C)	26.1	26.5	29.5	28.5	30.5
4	pH	7.5	7.6	8.2	8.1	8.5
5	Conductivity (mmhos/cm²)	1.04	1.12	1.26	1.24	1.95
6	Turbidity	20	20.6	27	44	72
7	Dissolved oxygen (mg/L)	10.8	9.6	5.8	5.4	4.9
8	Total organic carbon(mg/L)	39	41	42	43.6	45.8
9	BOD (mg/L)	12	23	36	22	44
10	COD (mg/L)	123	128	148	136	162
11	Ammonical nitrogen (mg/L)	1.19	1.28	1.31	1.54	1.66
12	Organic nitrogen (mg/L)	0.96	1.01	1.04	1.34	1.7
13	Nitrate nitrogen (mg/L)	0.03	0.08	0.24	0.68	0.98
14	Nitrite nitrogen (mg/L)	0.09	0.21	0.26	0.47	0.55
15	Total grease and oil (mg/L)	63	87	104	116	182

Table 2: Comparison of effluent quality with standards.

S.N	Parameters	Effluent	ISI Standards	TWQV	ISI Standards for waste water Irrigation	
					Desired	Objectionable
1	Colour	Yellow to blackish brown	Nil			Brown
2	Odour	Oily & phenolic	Nil		Normal	Obnoxious
3	Temperature (ºC)	18.3-32.9	<40			
4	pH	7.2-9.66	6.5-8.5	7.9	6.5-8.2	>6.5->8.2
5	Conductivity (mmhos/cm²)	0.73-1.95	<1.0		<1.0	>1.5
6	BOD (mg/L)	12.0-74.0	<3.0-30.0	26	<500	>500
7	COD (mg/L)	64.0-204.0	<250	100		
8	Phenols (mg/L)	0.0-0.06	0.005	0.3-7.8		
9	Tar, Oil & Grease (mg/L)	0.013-0.516	<0.0001	0.008-0.014	<0.5	>0.1

TWQA: Treated waste quality values (Burroughs 1963 & Mckinney 1963), ISI: Standard Clause-ISI Part I (1981), US-EPA: Process design manual for land treatment of municipal wastewater, US Environment Protection Agency (1981).

site remained near to atmospheric temperature at 30.2°C. The pH of soil was 7.9. Electric conductivity was found to be 24 mmhos/cm before irrigation. The maximum conductivity range (20-27 mmhos/cm) was found at 1%. The minimum conductivity from 17-24 mmhos/cm was found at 0.05%. It was 18-25 mmhos/cm at 1%, 18-25 mmhos/cm at 0.5% and 16-23 mmhos/cm at control site. Moisture content of the soil varied considerably throughout the year at the study site. Maximum moisture content was observed in the month of August (17.372%), while minimum in the month of May (8.246%). The humus content of experimental soil was 0.25%, whereas control soil had a variation from 0.15-0.24%. Total organic carbon in soil was 0.17% before irrigation. The maximum TOC of 0.23% was observed at 0.05%, while minimum of 0.02% was at 1%.

The total nitrogen of the effluent ranged from 1.19-1.66 mg/L. In soil it was 0.02% before irrigation. The maximum nitrogen of 0.12-0.18% was observed at 1%, while minimum of 0.06-

Table 3: Qualitative and quantitative spectrum of hydrocarbons in refinery effluent and soil (after crop harvesting).

S.No.	Hydrocarbons	Initial	1%	0.50%	0.10%	0.05%
1	Unknown	Tr	-	-	-	-
2	C-12	5.08	-	-	-	-
3	C-13	3.38	1.5	-	-	-
4	Unknown	10.17	-	-	-	-
5	C-13	11.86	-	-	-	-
6	C-14	11.86	4.5	-	8	-
7	Unknown	3.38	-	-	-	-
8	C-14	-	1.5	-	-	-
9	C-15	8.47	4.5	3.57	8	-
10	C-15	-	3	Tr	-	-
11	C-16	3.38	11.3	3.57	12	-
12	C-17	23.17	60	3.57	16	Tr
13	C-18	-	Tr	21.42	32	Tr
14	C-19	6.78	3	3.57	8	Tr
15	C-20	1.69	4.5	28.5	8	10
16	C-21	1.69	3.7	Tr	8	20
17	C-22	3.38	1.5	Tr	Tr	30
18	C-23	5.08	Tr	-	Tr	20
19	C-24	-	Tr	-	Tr	10
20	C-25	-	-	14.28	-	10
21	C-26	-	-	21.42	-	Tr
22	C-27	-	-	-	-	Tr

Tr = Traces, (-) = absent

0.12% was at 0.05%. The soil nitrogen at control site ranged from 0.06-0.11%. The soil was found to have no grease and oil before irrigation. The maximum oil and grease content (0.02-0.08%) was found at 1%, while minimum (0.00-0.01%) at 0.05%. At control soil grease and oil content was nil.

In soil, after harvesting of crops, gas chromatographic analysis was performed and studies showed the presence of chromatographic peaks of alkanes from C-14 to C-27. In all, 14 hydrocarbons existed in effluent irrigated soils. At 0.1%, 12 hydrocarbons were reported which ranged from C-13 to C-27. The concentration of C-17 was maximum, i.e. 86.4% and rest were recorded in traces only. At 1%, 11 hydrocarbons appeared from C-17 to C-27 with higher concentration.

At 0.5%, 11 hydrocarbons appeared ranging from alkanes C-14 to C-24, maximum concentration being of C-17 and C-18. At 0.05%, 8 hydrocarbons appeared ranging from alkanes C-20 to C-27 (Fig 1. and Table 3).

The gas chromatographic analysis revealed that the main hydrocarbons in diesel are saturated and unsaturated aliphatic hydrocarbons and a few unknown ones. In diesel 22 hydrocarbons were recorded. The gas chromatograms and qualitative analysis of the hydrocarbons are shown in Fig. 4 and Table 5. Overall 12 chromatographic peaks corresponding to n-alkanes of carbon length C-12 to C-22 were encountered.

Besides, alkenes C-19 to C-22 and few unknown hydrocarbons were also noticed. In soil after harvesting of crops, gas chromatographic analysis was performed and studies showed the presence of chromatographic peaks of alkanes from C-6 to C-22, and alkenes C-6 to C-11. At 9.1%, 11 hydrocarbons were reported which ranged from C-6 to C-22. In 1%, 18 hydrocarbons appeared from

Table 4: Physico-chemical characteristics of soil irrigated with different irrigants containing refinery effluents.

Concentration	1%				0.5%				0.1%				0.05%				Control			
Days	0	20	40	60	0	20	40	60	0	20	40	60	0	20	40	60	0	20	40	60
Temp.(°C)	30.00 ± 0.341	26.14 ± 0.273	25.08 ± 0.214	24.58 ± 0.504	30.00 ± 0.141	26.96 ± 0.301	26.38 ± 0.214	25.10 ± 0.316	30.30 ± 0.141	28.08 ± 0.141	27.08 ± 0.214	27.10 ± 0.200	30.36 ± 0.185	28.22 ± 0.214	27.52 ± 0.248	27.38 ± 0.172	30.10 ± 0.648	30.10 ± 0.424	28.24 ± 0.185	27.58 ± 0.279
pH (-log)	8.50 ± 0.071	7.42 ± 0.026	6.47 ± 0.042	6.41 ± 0.361	8.54 ± 0.053	7.43 ± 0.033	6.45 ± 0.035	6.21 ± 0.017	8.82 ± 0.052	8.45 ± 0.037	8.88 ± 0.445	7.87 ± 0.042	8.86 ± 0.034	8.47 ± 0.042	8.20 ± 0.029	8.12 ± 0.172	8.90 ± 0.255	8.64 ± 0.042	8.46 ± 0.036	8.45 ± 0.041
Conductivity (mmhos / cm²)	0.23 ± 0.017	0.22 ± 0.014	0.21 ± 0.017	0.16 ± 0.014	0.24 ± 0.014	0.22 ± 0.014	0.22 ± 0.014	0.17 ± 0.014	0.24 ± 0.019	0.25 ± 0.014	0.23 ± 0.014	0.18 ± 0.017	0.26 ± 0.010	0.25 ± 0.015	0.24 ± 0.019	0.18 ± 0.014	0.28 ± 0.032	0.27 ± 0.007	0.26 ± 0.010	0.20 ± 0.014
Moisture (%)	14.4 ± 0.013	13.42 ± 0.007	12.17 ± 0.121	12.33 ± 0.275	14.42 ± 0.006	13.42 ± 0.003	12.22 ± 0.004	12.28 ± 0.355	15.52 ± 0.006	14.42 ± 0.006	13.22 ± 0.010	13.09 ± 0.039	16.43 ± 0.002	15.30 ± 0.002	14.22 ± 0.005	14.21 ± 0.003	17.37 ± 0.010	16.45 ± 0.008	15.32 ± 0.005	14.22 ± 0.002
Humus (%)	0.16 ± 0.014	0.15 ± 0.017	0.14 ± 0.017	0.17 ± 0.010	0.16 ± 0.014	0.15 ± 0.014	0.14 ± 0.012	0.18 ± 0.014	0.20 ± 0.021	0.19 ± 0.014	0.15 ± 0.014	0.09 ± 0.014	0.22 ± 0.014	0.20 ± 0.014	0.15 ± 0.014	0.10 ± 0.014	0.24 ± 0.014	0.22 ± 0.014	0.20 ± 0.010	0.15 ± 0.010
Total organic carbon(mg/l)	0.20 ± 0.014	0.11 ± 0.010	0.06 ± 0.014	0.02 ± 0.012	0.22 ± 0.017	0.12 ± 0.014	0.06 ± 0.014	0.03 ± 0.010	0.23 ± 0.019	0.14 ± 0.014	0.06 ± 0.017	0.06 ± 0.014	0.23 ± 0.014	0.15 ± 0.007	0.07 ± 0.014	0.08 ± 0.014	0.28 ± 0.014	0.25 ± 0.010	0.20 ± 0.014	0.15 ± 0.014
Nitrogen (%)	0.11 ± 0.014	0.09 ± 0.014	0.07 ± 0.010	0.06 ± 0.014	0.12 ± 0.019	0.09 ± 0.014	0.08 ± 0.014	0.07 ± 0.010	0.14 ± 0.014	0.12 ± 0.014	0.10 ± 0.014	0.08 ± 0.017	0.15 ± 0.010	0.15 ± 0.014	0.12 ± 0.014	0.10 ± 0.014	0.18 ± 0.014	0.16 ± 0.009	0.16 ± 0.010	0.12 ± 0.014
Oil & Grease (%)	0.07 ± 0.014	0.05 ± 0.014	0.02 ± 0.008	0.01 ± 0.008	0.06 ± 0.017	0.04 ± 0.010	0.04 ± 0.010	0.02 ± 0.007	0.05 ± 0.012	0.05 ± 0.007	0.03 ± 0.007	0.02 ± 0.005	0.04 ± 0.040	0.01 ± 0.006	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000

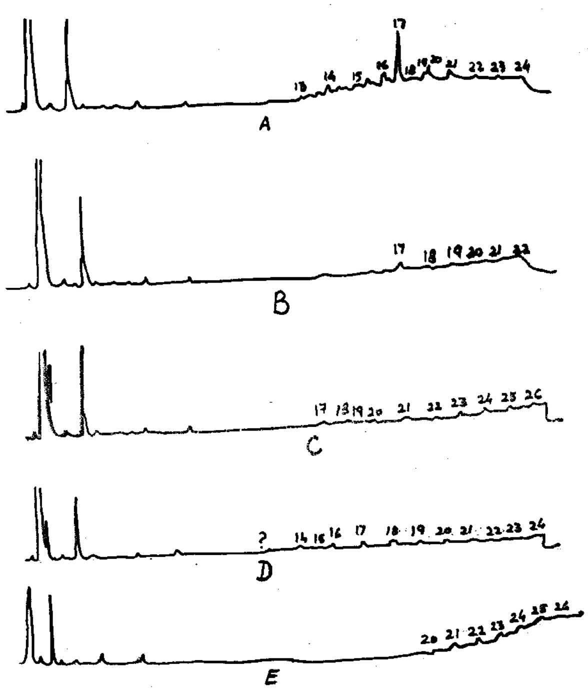

Fig. 1: Gas chromatographs showing the different hydrocarbons in refinery effluent and soils irrigated with different dilution after crop harvesting.

C-6 to C-22 and their concentrations were higher. At 0.05%, 12 hydrocarbons appeared from C-10 to C-18, maximum concentration being of C-16 and C-17. In pot with 0.05% diesel, 11 hydrocarbons appeared from C-6 to C-18.

Impact of oil pollution and refinery effluents on germination of test crops: Germination is affected by many factors. It was found that in case of the soil without any addition of refinery effluent, the germination percentage in *Lens esculenta* was 88.80 (Table 6). The seedlings emerged during 3-6 days but delayed to 9 days in effluent irrigated soil. When refinery effluent was added to irrigation water (0.05%), the percentage of germination increased in comparison to the 0.1%. After this, when more concentrated refinery effluent was applied, there was no significant difference from the percentage at 0.05%. When soil was irrigated with 0.05% and 1% of refinery effluent, the germination percentage was 88.0, higher than the control, and 30, 40, 36.60 and 91.40 higher than the treated effluent, i.e. 0.1%, 0.5%, 1.0% respectively at 0.05%. Similarly there was decrease in the germination percentage in the soil treated with refinery effluent at 0.05%. This decrease was 63.0 and 91.80, and 60.0 and 87.20 respectively. There was also a significant change within the germination recorded at 0.05% and 0.1% but in comparison to the control decrease was 88.0 and 30.40.

It was found that in case of soil without diesel, the germination percentage in *Lens esculenta* was 80.0. The seedling emerged between 4-7 days. When diesel was added with water to make dilutions 0.05, 0.1, 0.5 and 1.0%, the germination in these sets was 70.0, 50.0, 40.0 and 30.2 % respectively. When soil was irrigated with 1.0% diesel, the germination percentage drastically decreased in comparison to 0.05% and control.

It is evident from the results that the refinery effluent has adverse effect over the growth of the legume crops at higher concentration. The lower concentration 0.05% proved to be beneficial for germination. Hence, the refinery effluent can be utilized by the nearby farmers after proper dilution as a substitute for chemical fertilizers. Similar recommendations were also made by Vijayarengan & Lakshmanachary (1993) and Kannabiran & Progasam (1993).

Impact of refinery effluent on shoot growth of the test crop: The data on effect of refinery effluent on shoot growth are given in Tables 7 and 8. It is clear from the data given below that the number of leaves decreased with the increase in concentration of the effluents. The number of leaves was higher on the plants grown at lowest concentration of the effluent. The number of leaves at plot

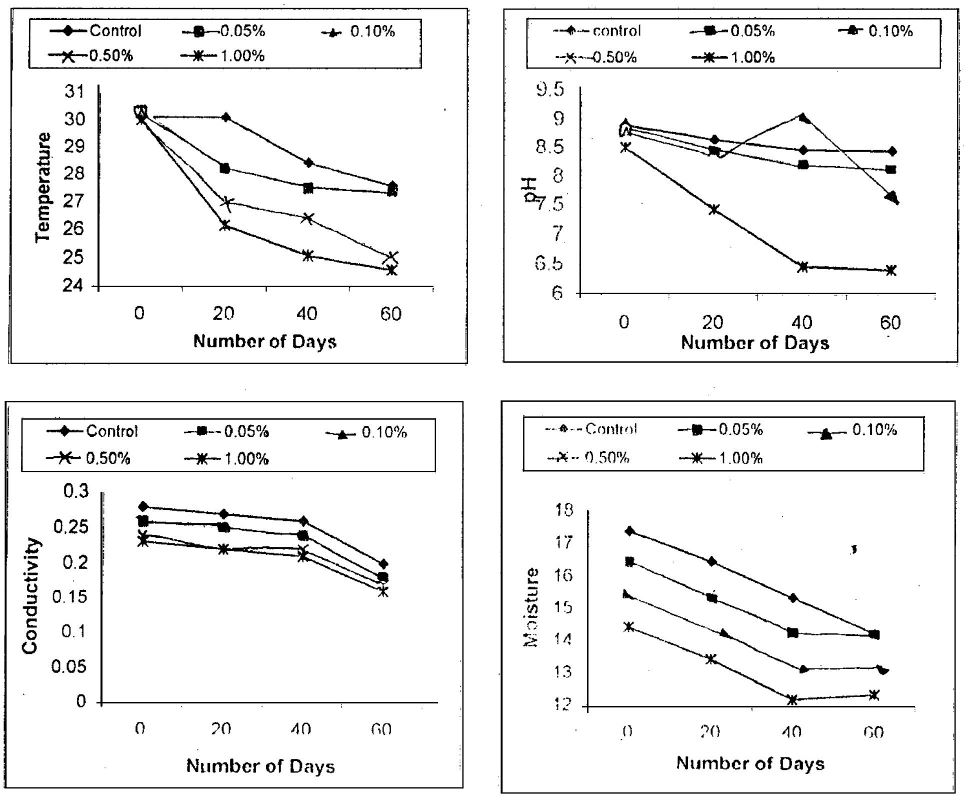

Fig. 2: Variation of soil properties with days at different concentrations of effluent.

without refinery effluent was 16, but on addition of effluent at 0.1%, 0.5% and 1%, the number of leaves decreased on average to 14.3, 12.2 and 10.2, respectively. At 0.05% it increased to 16.3. The shoot dry weight was maximum 7.5g at 0.05%, and minimum 3.3g at 1%. There was an increase in shoot length at 0.05% in comparison to non-effluent soil, while at other concentrations, it decreased gradually. At non-effluent irrigated soil, the shoot length was 43cm. It increased to 43.8cm when soil was irrigated with 0.05% effluent concentration, but decreased to 32.9cm, 23.4cm and 20.8cm at 0.1, 0.5 and 1% effluent containing soil.

The number of leaves in the pots without diesel was 14, but on addition of diesel at 0.05, 0.1, 0.5 and 1% the number of leaves were 14.8, 12.6, 12 and 11.4, respectively. In non-diesel irrigated soil the shoot length was 28.8cm, but it decreased gradually as the dose of the diesel was increased. It decreased to 28.4cm at 0.05%, 25cm at 0.1%, 22.8cm at 0.5% and 17.9cm at 1%. The shoot dry weight was maximum (4.1g) at 0.05%, and minimum (2.8g) at 1%.

The seedling height showed a gradual reduction with increase in the petroleum hydrocarbon concentration. The reduction in seedling height may be due to high TDS, COD and presence of heavy metals in the waste.

Impact of refinery effluent on root growth of test crop : The data on effect of refinery effluent on root growth are given in Tables 9 and 10. It was observed that in the plot without any addition of

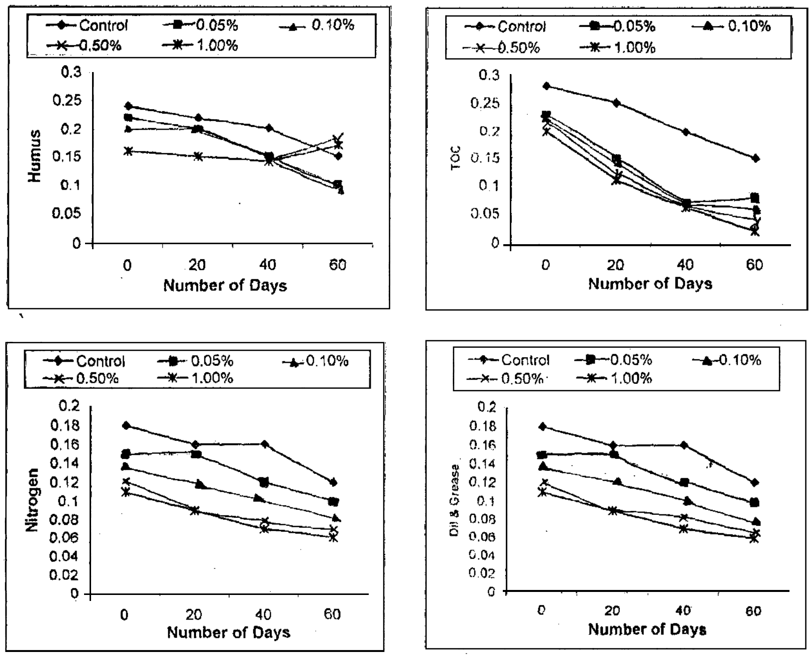

Fig. 3: Variation of soil properties with days at different concentrations of effluent.

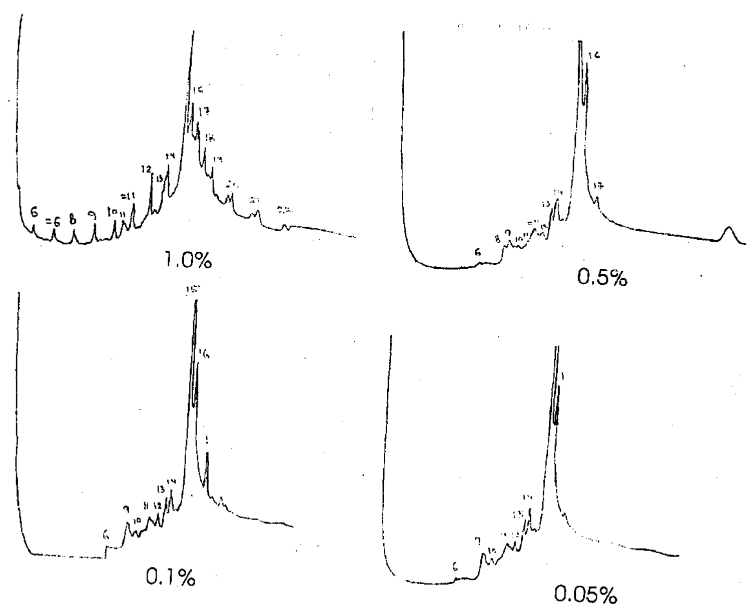

Fig. 4: Gas chromatographs showing the diferent hydrocarbons in diesel and soils irrigated with different dilution after crop harvesting.

refinery effluent, the dry weight of the roots was 4.5 g/plant with root length of 13.2 cm. At 0.05% dilution the dry weight and root length increased to 4.6g/plant and 13.6cm, respectively. The dry weight was 3.5, 3.0 and 2.6 g/plant at 0.1%, 0.5% and 1% dilution respectively.

The root length was 11.0, 9.7 and 9.5 cm at 0.1%, 0.5% and 1% dilution respectively. The number and size of nodules per plant were found to be too much affected by addition of refinery effluent. The average number of nodule was 6.1 with control, which decreased to 4.16 at 1%, 5.64 at 0.5% and 5.68 at 0.1%, but increased to 6.4 at 0.05%.

In pot experiment, it was observed that without any addition with diesel the dry weight of the root was 2.3 g/plant with root length of 6.2 cm. At 0.05% dilution the dry weight and root length increased to 2.5g/plant and 6.5cm, respectively. The dry weight was 2.1, 1.8 and 1.4 g/plant at 0.1%, 0.5% and 1% dilution respectively. The root length was 5.6, 4.7 and 4.2 cm at 0.1%, 0.5% and 1% dilution respectively. The average number of nodules was 4.25 with control but decreased to 2.2 at 1%, 3.1 at 0.5% and 4.20 at 0.1%, and increased to 5.5 at 0.05%.

Table 5: Qualitative and quantitative spectrum of hydrocarbons in diesel recovered from soil after plant harvesting.

Hydrocarbons	1.0%	0.5%	0.1%	0.05%
Unknown	-	Tr	0.01	-
C-6	0.246	0.382	0.376	0.1146
C-6	0.415	-	-	Tr
C-7	-	-	Tr	-
C-8	0.548	1.483	-	-
C-9	0.047	2.659	1.063	2.906
Unknown				
C-10	0.178	1.207	0.507	1.753
C-11	0.734	0.603	0.735	3.632
C-11	0.923	0.747	-	-
C-12	2.075	2.326	0.546	1.231
C-13	2.144	2.034	0.614	3.236
C-14	1.765	3.237	0.833	5.516
C-15	2.502	29.815	3.027	19.594
Unknown			0.478	
C-16	9.675	4.024	3.772	24.062
C-17	4.495	5.998	1.341	15.361
C-18	7.834	-	0.535	5.669
C-19	4.998	-	-	Tr
C-20	2.969	Tr	-	-
C-21	3.136	-	-	-
C-22	0.664	-	-	-

Tr = Traces, (-) = Absent

Table 6: Germination percentage of crops in field and pot under irrigation with various concentrations.

Lentil	1%	0.5%	0.1%	0.05%	Control
Field	45.00 ± 1.414	53.00 ± 1.789	66.60 ± 1.625	88.80 ± 1.470	85.40 ± 2.332
Pot	32.20 ± 1.312	40.00 ± 1.421	50.00 ± 1.812	70.00 ± 1.516	80.00 ± 1.419

Table 7: Effect of the concentration of diesel on shoot growth of *Lens esculenta* in pot experiment.

Lentil	1%	0.5%	0.1%	0.05%	Control
Shoot Length	17.85 ± 1.281	22.80 ± 0.251	25.00 ± 0.582	28.40 ± 0.364	28.85 ± 0.984
No. of Leaves	11.14 ± 0.721	11.95 ± 0.781	12.56 ± 1.121	14.80 ± 1.305	13.95 ± 1.164
Shoot dry weight	2.80 ± 0.375	3.10 ± 0.082	3.80 ± 0.420	4.10 ± 0.665	4.20 ± 0.775

Table 8: Effect of the concentration of refinery effluent on shoot growth of *Lens esculenta* in field experiment.

Lentil	1%	0.5%	0.1%	0.05%	Control
Shoot Length	20.80 ± 3.281	23.36 ± 2.209	32.96 ± 5.760	43.88 ± 1.330	43.00 ± 1.280
No. of Leaves	10.20 ± 2.215	12.18 ± 1.360	14.30 ± 1.955	16.32 ± 2.311	15.80 ± 3.040
Shoot dry weight	3.32 ± 0.255	4.08 ± 0.495	6.86 ± 1.464	7.46 ± 0.402	7.27 ± 0.518

Table 9: Effect of the concentration of refinery waste on root growth of *Lens esculenta* in field experiment.

Lentil	1%	0.5%	0.1%	0.05%	Control
Root Length	9.56 ± 1.941	9.75 ± 1.046	11.09 ± 0.232	13.60 ± 1.194	13.28 ± 0.186
No. of Nodules	4.16 ± 0.774	5.64 ± 1.242	5.68 ± 0.700	6.40 ± 0.704	6.12 ± 1.001
Root dry weight	2.67 ± 0.371	3.00 ± 0.381	3.56 ± 0.410	4.54 ± 0.475	4.50 ± 0.347

Table 10: Effect of the concentration of diesel on root growth of *Lens esculenta* in pot experiment.

Lentil	1%	0.5%	0.1%	0.05%	Control
Root Length	4.20 ± 0.774	4.70 ± 0.460	5.60 ± 0.701	6.50 ± 0.706	6.20 ± 1.001
No. of Nodules	2.20 ± 0.137	3.10 ± 0.082	4.20 ± 0.775	5.55 ± 1.537	4.25 ± 0.776
Root dry weight	1.40 ± 0.310	1.80 ± 0.332	2.15 ± 0.124	2.50 ± 0.275	2.30 ± 0.245

DISCUSSION

The effluent at studied concentrations has delaying effect on germination. This may be due to the toxic effect of the effluent. The rate of germination is low at higher concentration. Inhibition of seed germination to a higher concentration of the effluent may be due to high levels of dissolved solids, which enrich the salinity and conductivity of the observed solute by the seed before germination (Neelam & Sahai 1988, Ramesh Kumar et al. 1990, Gautam et al. 1992).

The seedling height showed a gradual reduction with increase in the petroleum hydrocarbon concentration. The reduction in seedling height may be due to high TDS, COD and presence of heavy metals in the waste. The heavy metals of most concern are Cu, Pb, Zn, Cd, Cr. If these are present in large amounts in the source, they are potentially toxic to plant and animals (Luo & Rimmer 1995). The heavy metals present in the effluents at low concentration stimulate seedling growth whereas higher concentrations inhibit the growth. It is evident from the result that the refinery effluent has adverse effect on the growth of the *Lens esculenta* at higher concentrations. The lower concentration of 0.05% proved to be beneficial for germination and growth.

Reduced plant growth under petroleum refinery effluent has been attributed mainly to altered photosynthesis and changed pattern of translocation of photosynthesis. The toxicity of heavy metals affect the photosynthesis by decrease of photosynthesis per unit leaf area. The results of the present investigation is comparable with report of Nag et al. (1981). Nutrients such as nitrogen, potassium, calcium, magnesium etc., present in the diluted effluent, in lower concentrations increase growth of the plants, however at higher concentration of the effluent the said nutrients are raised to the levels, which probably become toxic resulting in inhibition of root and shoot growth.

It was found that the performance of *Lens esculenta* as assessed in terms of shoot length, leaf number, shoot dry weight, root length, root nodule number and root dry weight was adversely affected by the effluent of Mathura refinery at low dilution.

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