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Impact of Fuel Oil on the Freshwater Alga *Selenastrum capricornutum*

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Some attempts have been made to assess the impact of oil and hydrocarbons on algae (O'Brein and Dixon 1976. Vandermeulen and Ahern 1976, Morales-Loo and Goutz 1990). Petroleum hydrocarbons in the aquatic environment could have subtle effects on the relative abundance of algal species, inhibiting growth of some sensitive species and promoting growth of the tolerant ones (Morales-Loo and Goutz 1990, Herman et al. 1991).

Selenastrum capricornutum is one of the most sensitive species of unicellular green algae and is a good indicator or bioassay organism for petroleum hydrocarbon exposure (Coffey et al. 1976, Herman et al. 1991). Algal bioassays are ecologically significantly since algae are the dominant primary producers in aquatic environment.

The present study investigated the impact of fuel oil on *S. capricornutum.* Chlorophyll (a) content (Chl a), growth rate (u/d), effective concentration (EC50); carbohydrate and protein contents were chosen as toxicity end points.

MATERIALS AND METHODS

The green alga *S. capricornutum* was obtained from the algal culture collection, University of Texas, Austin (USA), and grown in nutrient medium according to ASTM (1986). The test organism was in the logarithmic phase of growth when introduced to the standard algal culture media. The culture was incubated at room temperature (24 ± 2°C) under continuous illumination (2000 lux). Prior to conducting the bioassay, the organism was separated from the medium by centrifugation and washed with distilled water (Epstein and Schultz 1965).

A reference fuel oil sample was obtained from EPA (USA) namely Api oil, No. 2, 38% aromatic. 1274; and used for the preparation of the water

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extract. The latter was prepared by adding 2g of oil to one liter of distilled water, followed by stirring for 24 hr. The aqueous mixture was transferred to a separatory funnel and shaken for a few minutes and left for phase separation. The aqueous layer was used as the test solution where 1 ml corresponds to 0.002g of the oil. The standard oil extract was added to the algal cultures to yield the concentration levels of 0.03, 0.07, 0.012 and 0.25 g/l. Each concentration was represented by three flasks. For each run three control flasks, free from fuel oil, represented the normal order of growth. All cultures were incubated for 10 d.

Algal growth was determined by measuring Chl a content according to APHA (1995). Growth rate was determined by the equation: $\mu/d = \ln (x_2 - x_1)/ t_2 - t_1$ where x_1 and x_2 are the log Chl a at time intervals t_1 and t_2. The effective concentration value (EC50), which caused 50% inhibition of growth, was determined according to Finney (1971) using Chl a content at the exposure time intervals for fuel oil concentration tested.

Preliminary test indicated that *S. capricornutum* reached the logarithmic growth phase after 3 days of incubation, thereafter the culture passed to the stationary phase. Consequently, the time intervals selected for the determination of Chl a content and growth rate were 1, 3, 5, 6, 7, 8 and 10 days to represent the general pattern of growth.

Extraction of total carbohydrates from algal cells was carried out according to Myklestad and Haug (1972). Glucose content of the carbohydrate extracts was determined spectrophotometrically at 490 nm (Berkin Elmer, Lambda 3). Total protein content of algal cells was determined according to Lowery et al. (1951) as modified by Quian and Wang (1984) using a reference reagent of peptone as standard protein. Absorbance was measured at 700 nm using a spectrophotometer.

Results of Chl a, total carbohydrate and protein contents were subjected to statistical analysis according to Duncan's multiple range test (Duncan 1955). Correlation coefficient was derived according to Snedecor and Cochran (1980)

RESULTS AND DISCUSSION

The present study revealed that water extracts of fuel oil induced significant changes in Chl a content of treated cultures (Table 1). There was a general observed trend with Chl a content decreasing as the fuel oil

Table 1 Changes in chlorophyll (a) content (μg/l) of *S. capricornutum* in presence of fuel oil (initial Chl (a) content 69.2 μg/l).

Time day	1st	3rd	5th	6th	7th	8th	10th
Concentration of Fuel Oil (g/l)	Chl (a)	Chl (a)	Chl (a)	Chl (a)	Chl (a)	Chl (a)	Chl (a)
0.00	87.109^{f}	546.600^{e}	1099.500^{e}	1359.522^{e}	1421.320^{e}	1499.210^{e}	1399.210^{b}
0.03	75.577^{e}	213.293^{d}	308.893^{d}	624.310^{c}	1124.151^{c}	1212.010^{b}	1308.336^{b}
0.07	58.480^{d}	126.903^{c}	267.661^{c}	767.217^{d}	1267.867^{d}	1386.097^{d}	1353.670^{b}
0.12	51.820^{c}	110.526^{b}	158.114^{b}	600.213^{b}	1114.613^{b}	1309.843^{b}	1365.078^{b}
0.25	45.815^{b}	75.570^{a}	74.555^{a}	90.420^{a}	106.059^{a}	133.016^{a}	482.860^{a}

* Average of three replicates
Mean followed by the same letter in each column are not significant at 5% level-according to Duncan's Multiple Range Test.

Table 2 Growth Rates ** of *S. capricornutum* treated with fuel oil derived at short time intervals.

Concentration of Fuel Oil (g/l)	Growth Rates Over Time (days)						
	0-1	1-3	3-5	5-6	6-7	7-8	8-10
0.00	0.230	0.918	0.349	0.212	0.044	0.530	-0.035
0.03	0.088	0.519	0.185	0.704	0.588	0.075	-0.038
0.07	-0.158	0.387	0.373	1.050	0.502	0.089	-0.012
0.12	-0.289	0.378	0.179	1.330	0.619	0.161	0.021
0.25	-0.412	0.250	-0.006	0.193	0.159	0.226	0.645
*r^2	0.961(S)	0.74(S)	0.647(S)	0.274(N.S)	0.289(N.S)	0.031(N.S)	0.052(N.S)

* r^2 = Correlation Coefficient of growth rate vs. treatment concentration
** = Measured as Chl (a) content
(N.S) = Not Significant at 5% level
(S) = Significant at 5% level

concentration in algal cultures increased. Maximum Chl a content of control algal culture (1499 µg/l) was attained after 8 days of incubation. In case of cultures treated with 0.03, 0.07 and 0.12 g/l fuel oil extracts, maximum Chl a contents were attained after 10 days of incubation and were not significantly different from the control, showing the ability of algal cells to recover.

Considering the growth rate equation, the rate could be calculated to represent growth attained within specific short time intervals (Table 2) or the net growth after an extended incubation period (Table 3). Maximum growth rate (µ max) of the control cultures was observed at the time interval of 1-3 d, whereas such rate was delayed, due to the application of 0.03, 0.07 and 0.12 g/l fuel oil extracts, to the time interval of 5-6 d. Values representing the net growth rates (Table 3) showed a similar impact of fuel oil treatments on algal growth as given in Table 2. However, values representing the net change in growth rates were always lower compared to the corresponding values recorded at short time intervals.

The correlation between applied fuel oil concentrations and net growth rate was significant at most time intervals investigated (Table 3). Growth rates derived at short-time intervals (Table 2) were not significantly correlated with the applied fuel oil concentration through out the exposure period from 1 to 10 d. Lack of significance may be attributed to the ability of algal cells to recover after variable exposure periods according to the applied fuel oil concentration (Table 1).

EC50 values attained by probit analysis, in terms of Chl a content, were 0.015, 0.014 and 0.156 g/l for the time interval of 3-5 and 7 d, respectively. The relative high value of 0.156 g/l given for 7 d exposure reflects the ability of algal cells to adapt to the inhibitory effects of fuel oil. According to Herman et al. (1991), the EC50 for growth inhibition of *S. capricornutum*, after 8 d exposure to a mixture of aromatic hydrocarbons (which represents the water soluble fraction of oil) was 22.7 mg/l.

Exposure of *S. capricornutum* to fuel oil extracts significantly decreased the carbohydrate and protein content of algal cells compared to control cultures (Table 4). The cell carbohydrate and protein contents progressively decreased with increasing fuel oil concentration.Herman et al. (1991) similarly reported that the ability of *S. capricornutum* to retain 14-C labelled photosynthates was diminished when the cells were exposed to aromatic hydrocarbons. Previous study by Tukaj (1987), using the green alga *Scenedesmus* sp. as a test organism, showed a decrease in Chl a

Table 3 Net change in growth rates ** of *S. capricornutum* treated with fuel oil.

Concentration of Fuel Oil (g/l)	Growth Rates Over Time (days)						
	0-1	0-3	0-5	0-6	0-7	0-8	0-10
0.00	0.230	0.689	0.553	0.496	0.432	0.384	0.301
0.03	0.088	0.375	0.229	0.367	0.398	0.358	0.294
0.07	-0.168	0.202	0.271	0.401	0.415	0.375	0.297
0.12	-0.289	0.156	0.165	0.360	0.397	0.368	0.298
0.25	-0.412	0.029	0.015	0.045	0.061	0.082	0.194
*r^2	0.961(S)	0.578(N.S)	0.676(S)	0.274(S)	0.856(S)	0.877(S)	0.948(S)

r^2 = Correlation Coefficient of growth rate vs. treatment concentration
(N.S) = Not Significant at 5% level
** = Measured as Chl (a) content
(S) = Significant at 5% level

Table 4 Effect of fuel oil on carbohydrate and protein contents of *S. capricornutum.*

Concentration of Fuel Oil (g/l)	Parameter**	
	Carbohydrate (mg/g d.wt)	Protein (mg/g d.wt)
0.00	149.34^{e}	5.26^{d}
0.03	105.00^{d}	3.89^{c}
0.07	73.91^{c}	3.54^{b}
0.12	29.70^{a}	3.43^{b}
0.25	8.71^{a}	2.07^{a}

* Mean followed by the same letter in each column are not significant at 5% level-according to Duncan's multiple range test.
** After seven days of incubation.

content, dry matter and number of cells in the treated cultures as the concentration of fuel oil was increased. The effects of petroleum hydrocarbons on the growth and the modification of certain physiological and biochemical processes were also reported by several investigators (Zachleder et al. 1983, Vandermeulen and Lee 1986). Fuel oil could also extend the period required for algae to adapt, delayed growth and leading to a relatively lower cell density (Soto et al. 1977, Dahl et al. 1983). Such findings give more support to our results where μ max values of *S. capricornutum* treated fuel oil culture were attained after 7 to 10 d of incubation versus control μ max values recorded after 3 d.

Oil refinery wastewater, conventionally treated, still contains about 20 mg/l total hydrocarbons and the new European standards will require less than 5 mg/l hydrocarbons (Elmaleh and Ghaffor 1996). The present study gives more support to the efforts leading to the control of water pollution by oil.

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